

Cleavage sites of endoproteases on the α -chain of GPIb

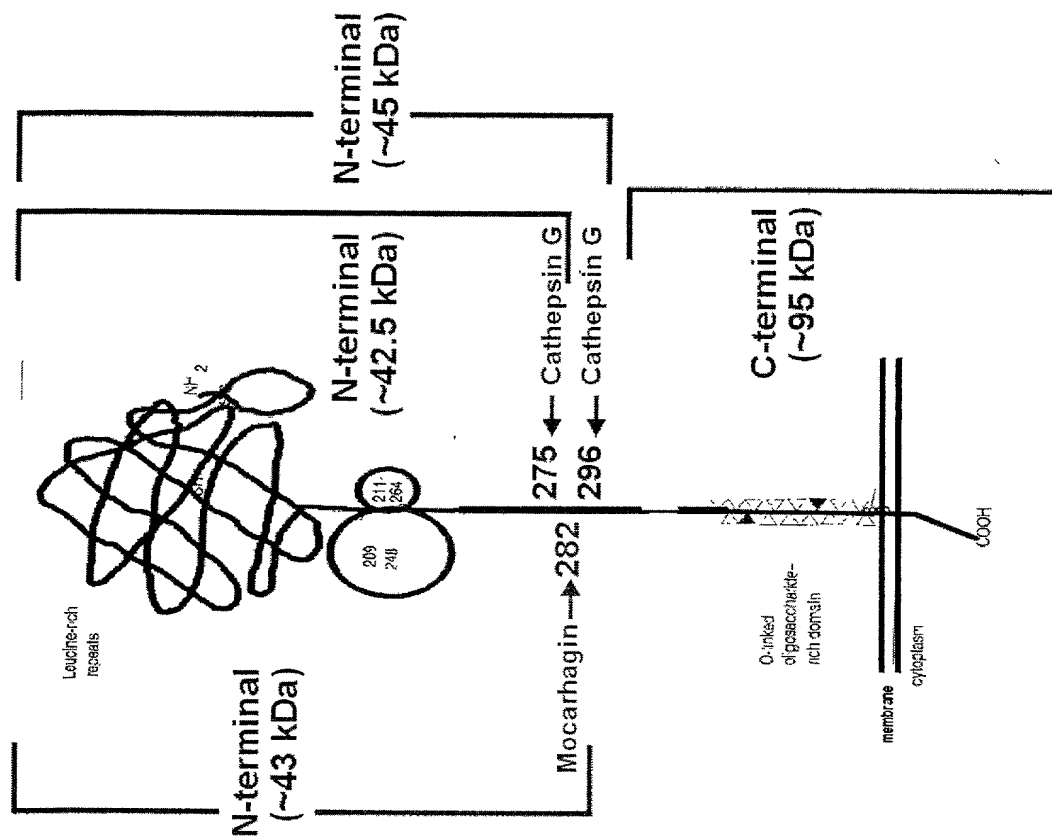
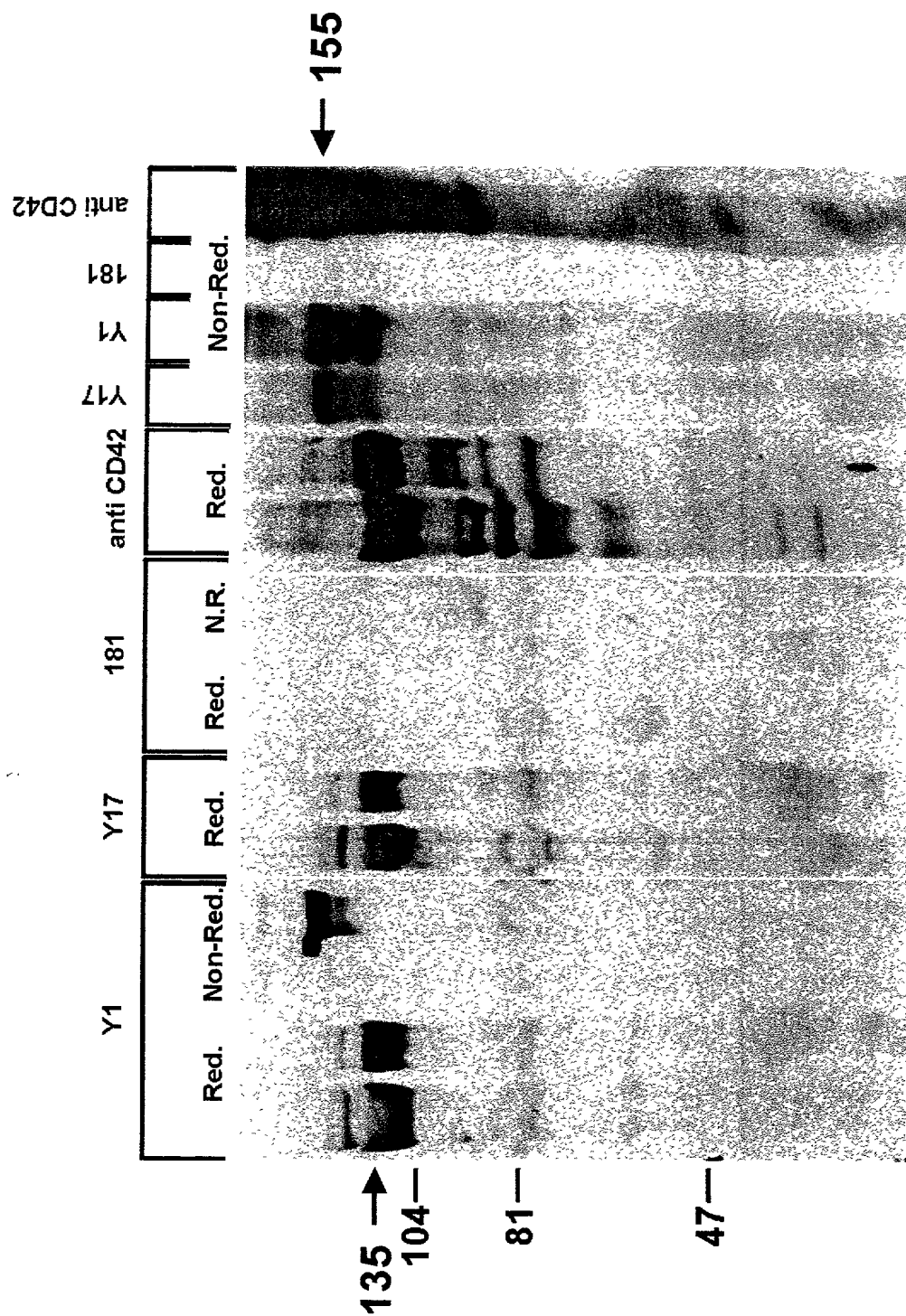


FIG. 1

FIG. 2

Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions

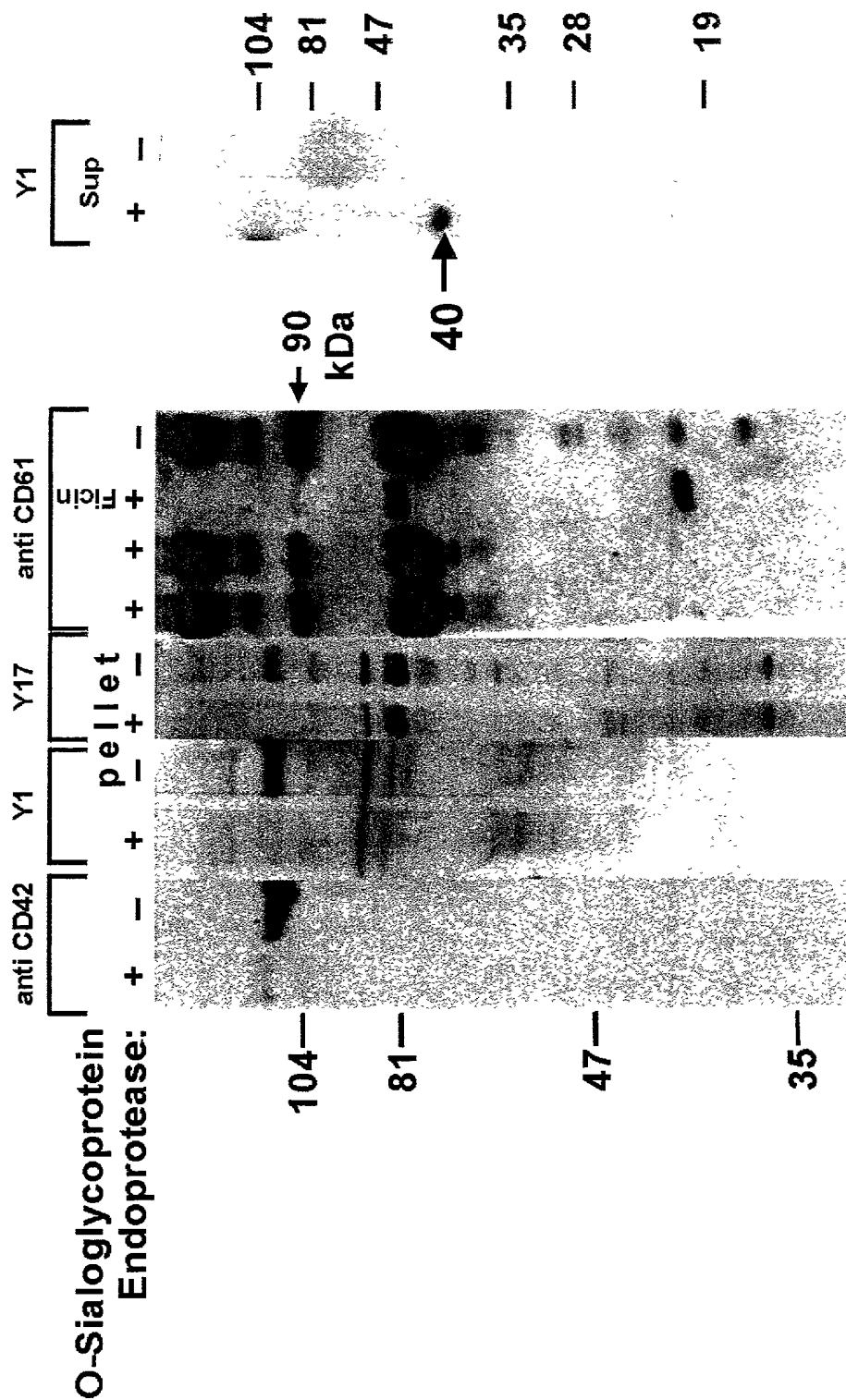


Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

FIG. 3

	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340 GP1b 1-480	- -	
Glycanase: N N+O	+ +	+++ +++
Proteases: Mocarhagin O-Sialo Peptidase Ficin Trypsine Elastase	++ (~40kD) ++ (~40kD) - ++ (~40kD) ++ (~40kD)	- - - - ++
Sulfatase (Aryl)		-/+

Cleavage of platelets GPIb by O-Sialoglycoprotein abolishes binding of both Y1 and Y17



Y1 and Y17 binds similar glycosylated fragments after cleavage by O-Sialoglycoprotein Endoprotease

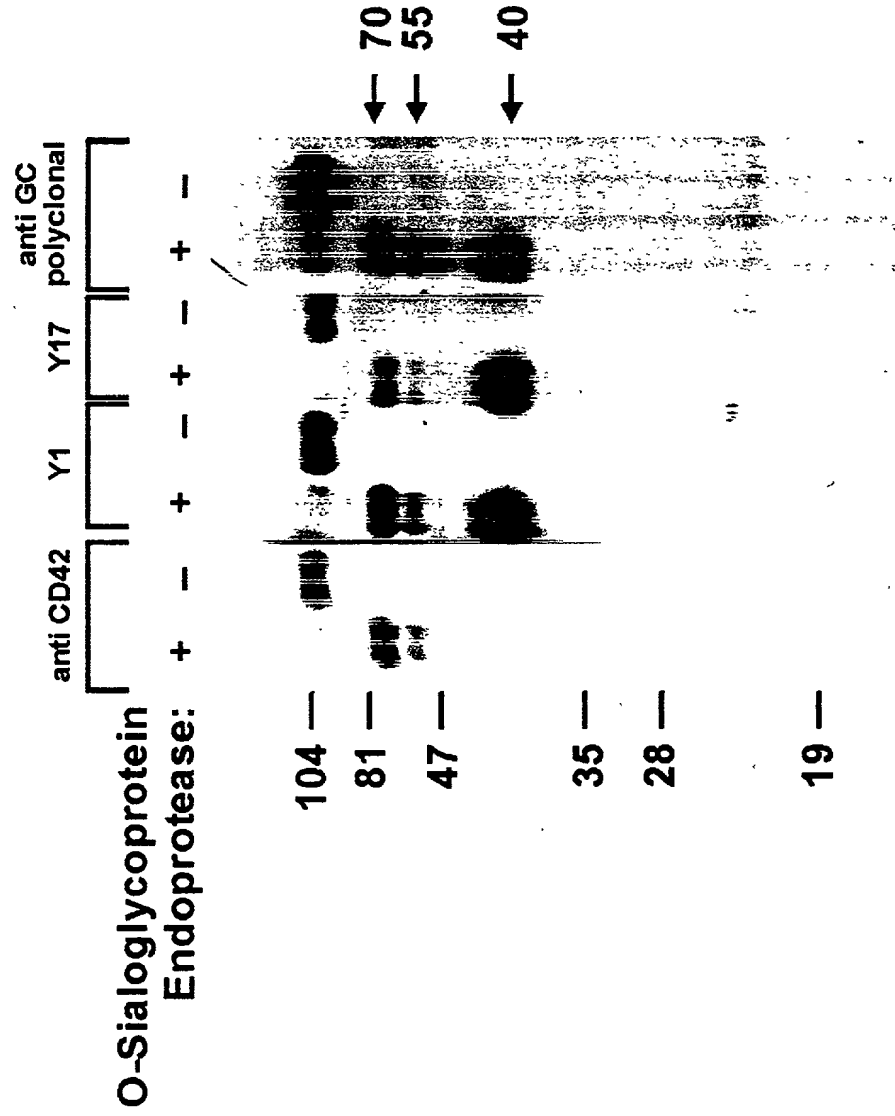
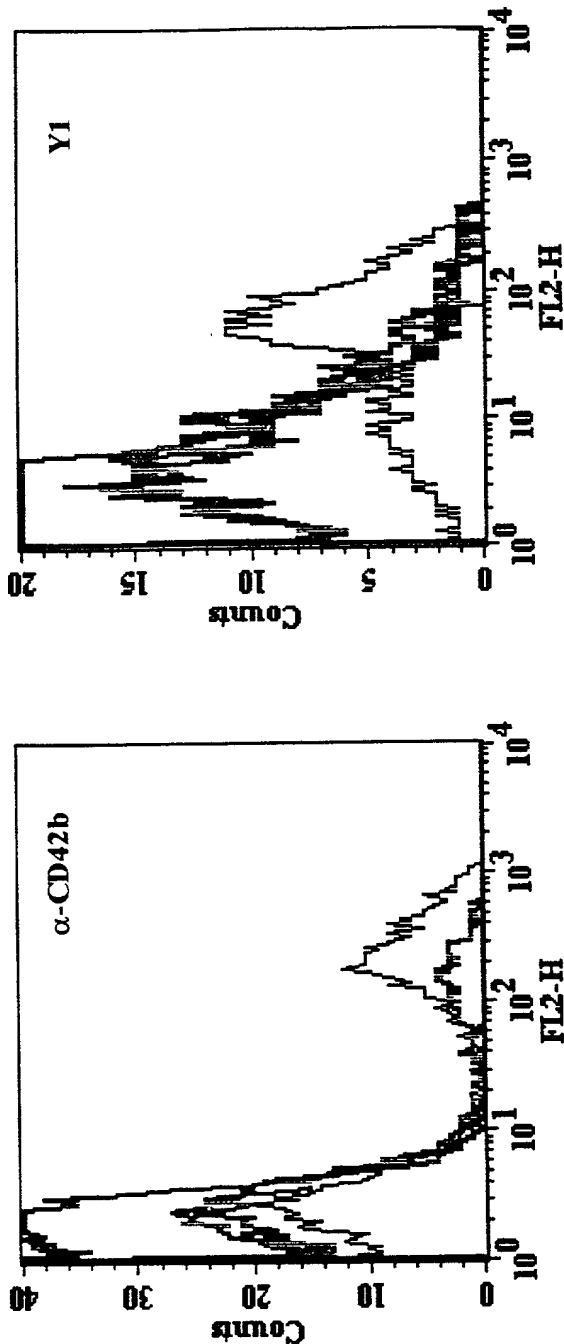


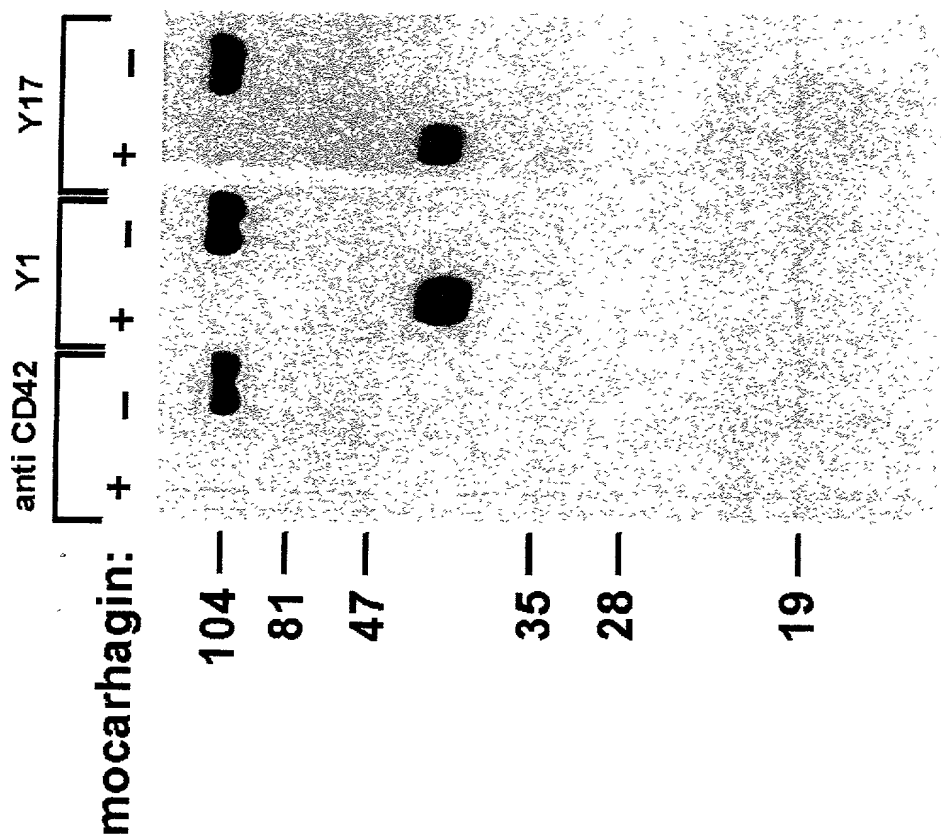
FIG. 5

Specific GPIIb Proteolysis Abolishes Y1 Binding to Platelets



Key Name Parameter G

- NON-TREATED PLATELETS
- O-SIALOGLYCOPROTEIN ENDO. (10 μg/ml)
- O-SIALOGLYCOPROTEIN ENDO. (50 μg/ml)
- FICIN (18 μg/ml)

[illegible]

104— 81— 47— 35— 28—

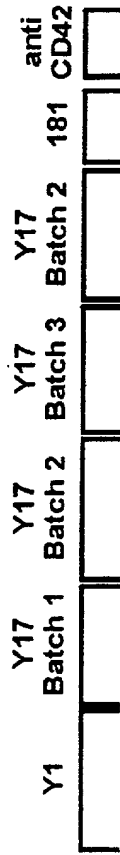


FIG. 10

Y1 and Y17 bind glycoprotein similar after cleavage by Ficin

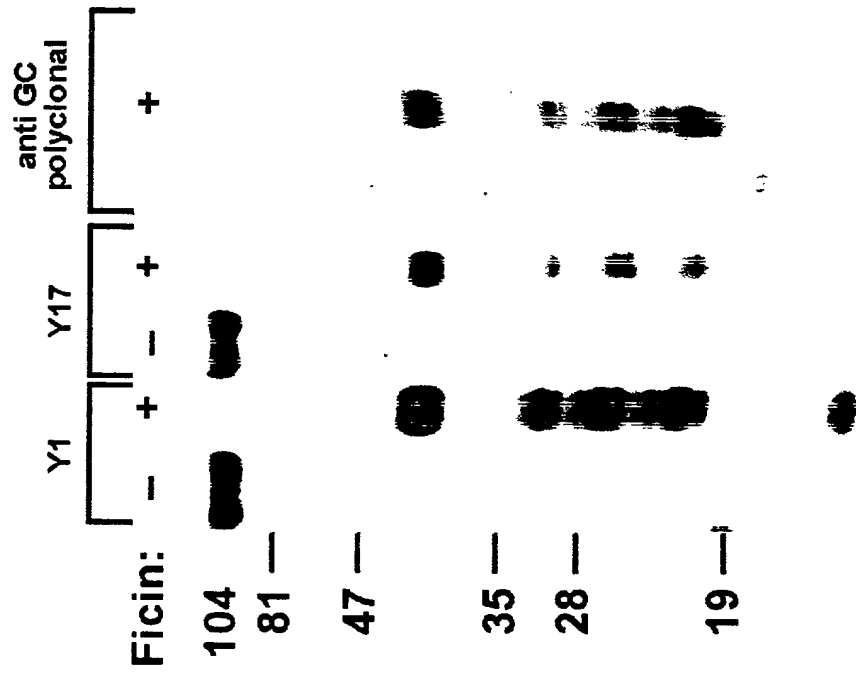
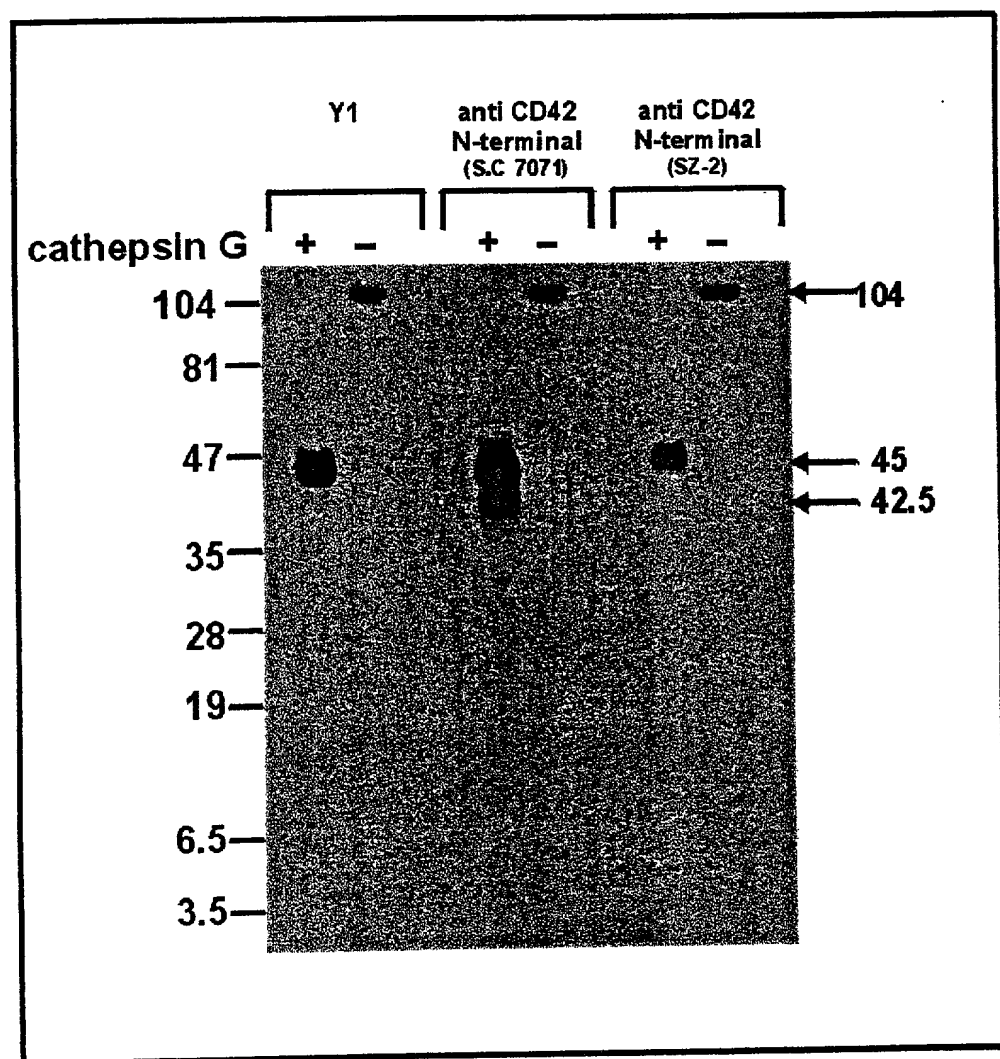


FIG. 11



Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIb fragment

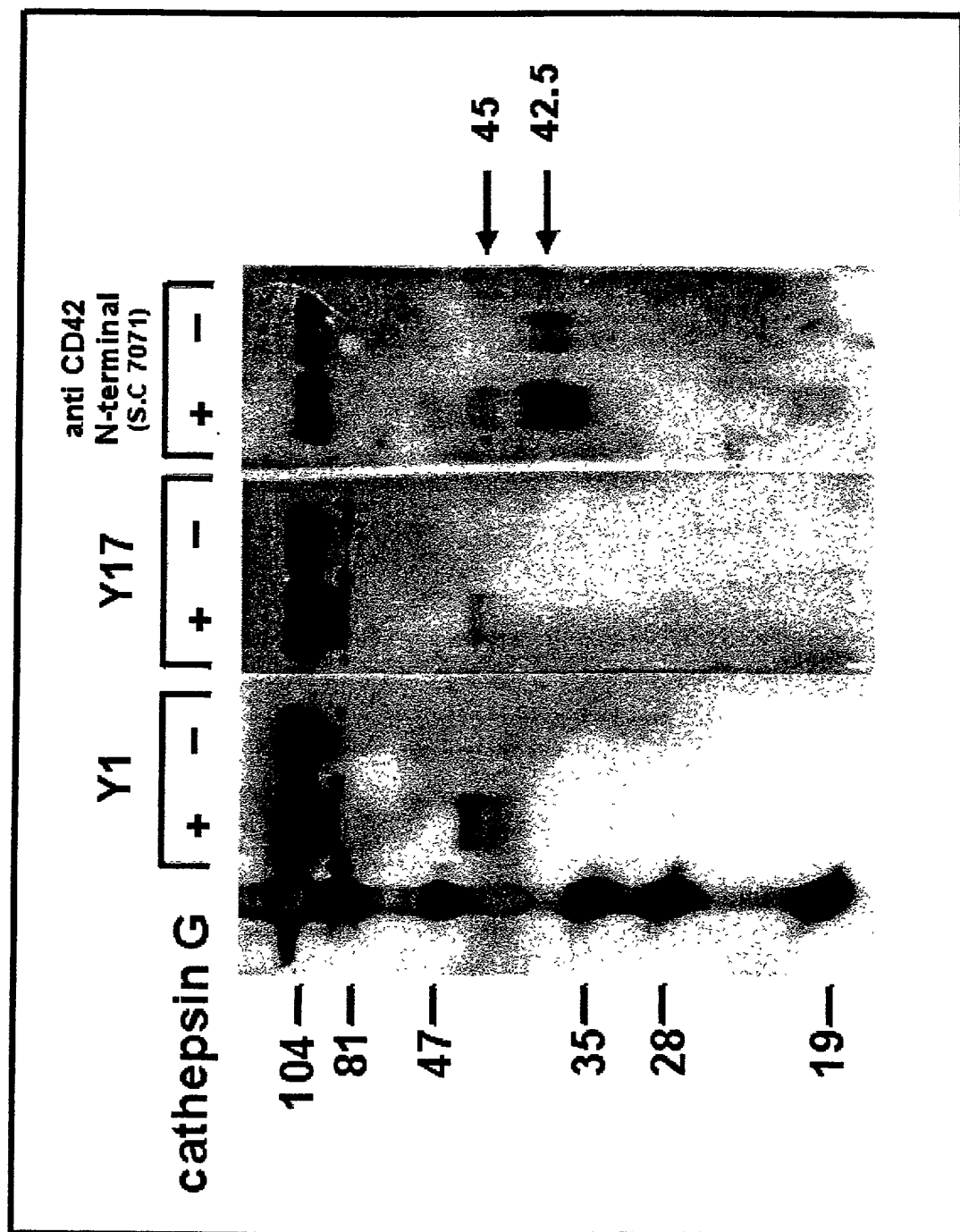


FIG. 13

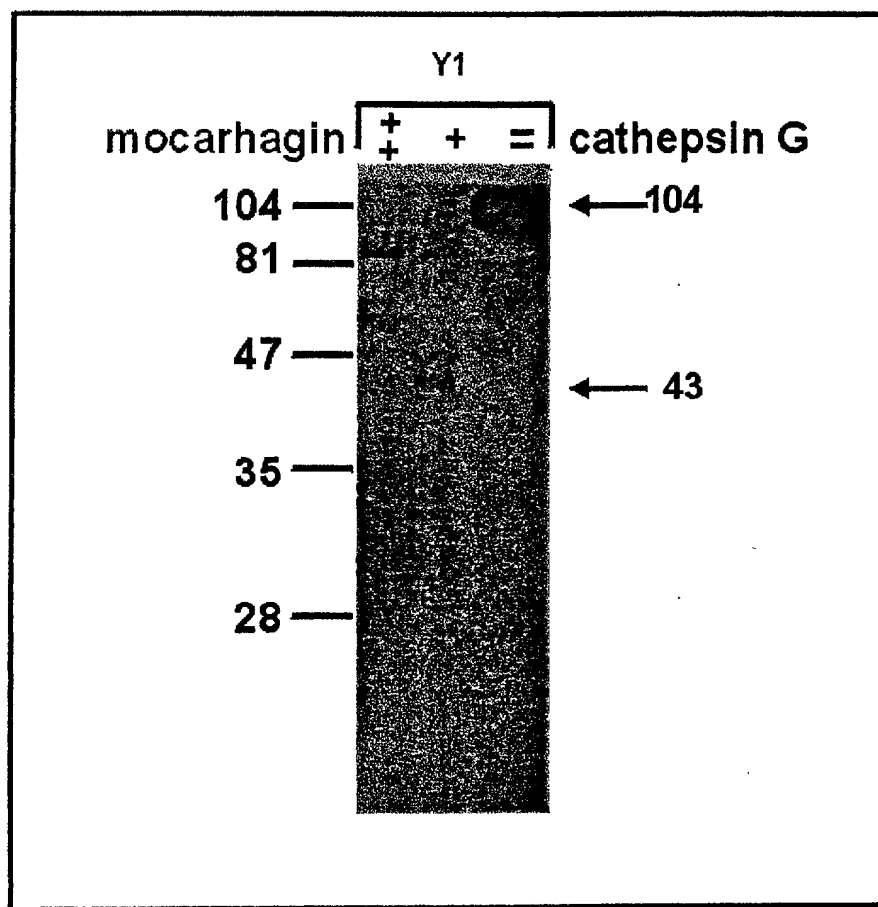


FIG. 14

Cleavage of washed platelets by mocarhagin and cathepsin G

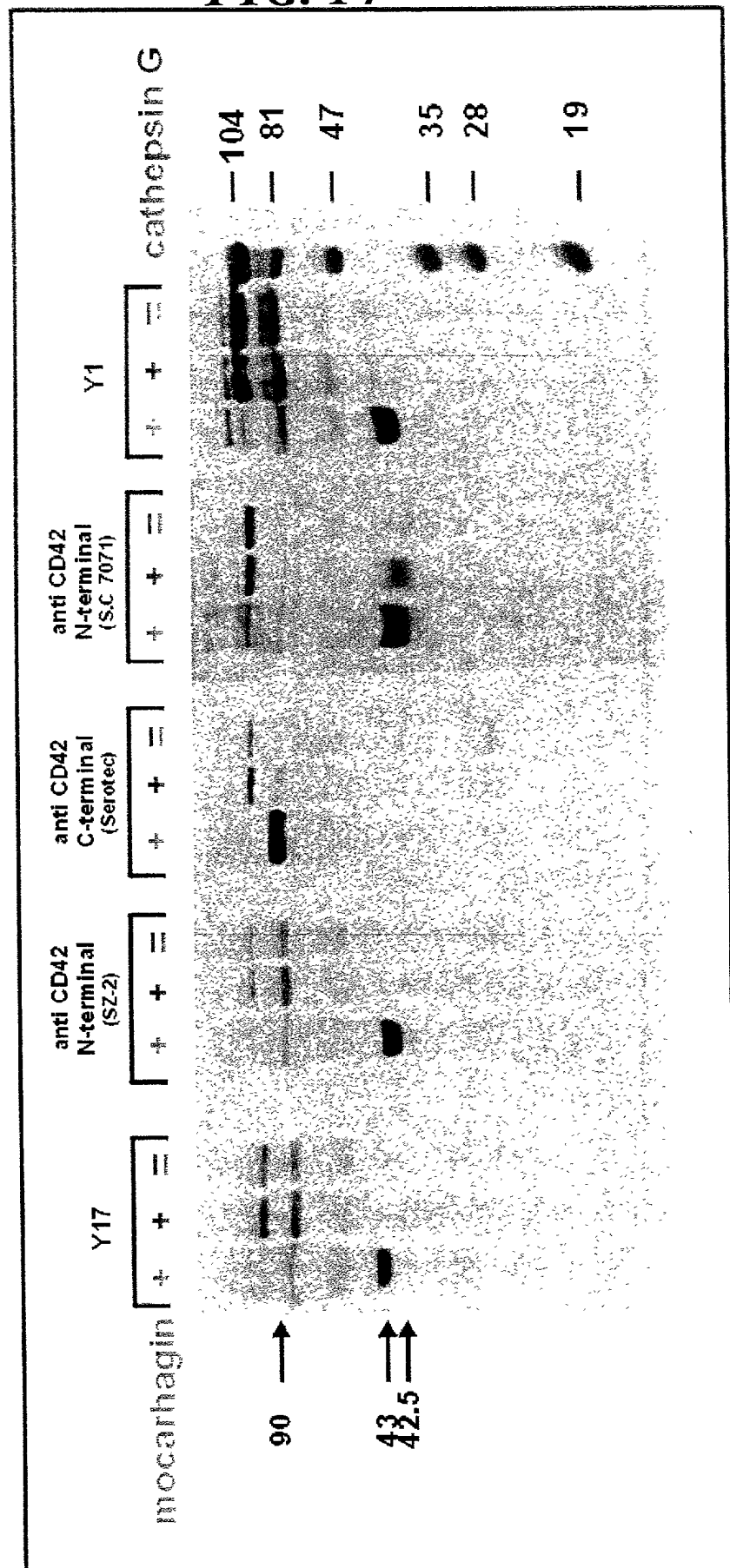


FIG. 15

Influence of Y1-scFv on platelets agglutination in washed platelets

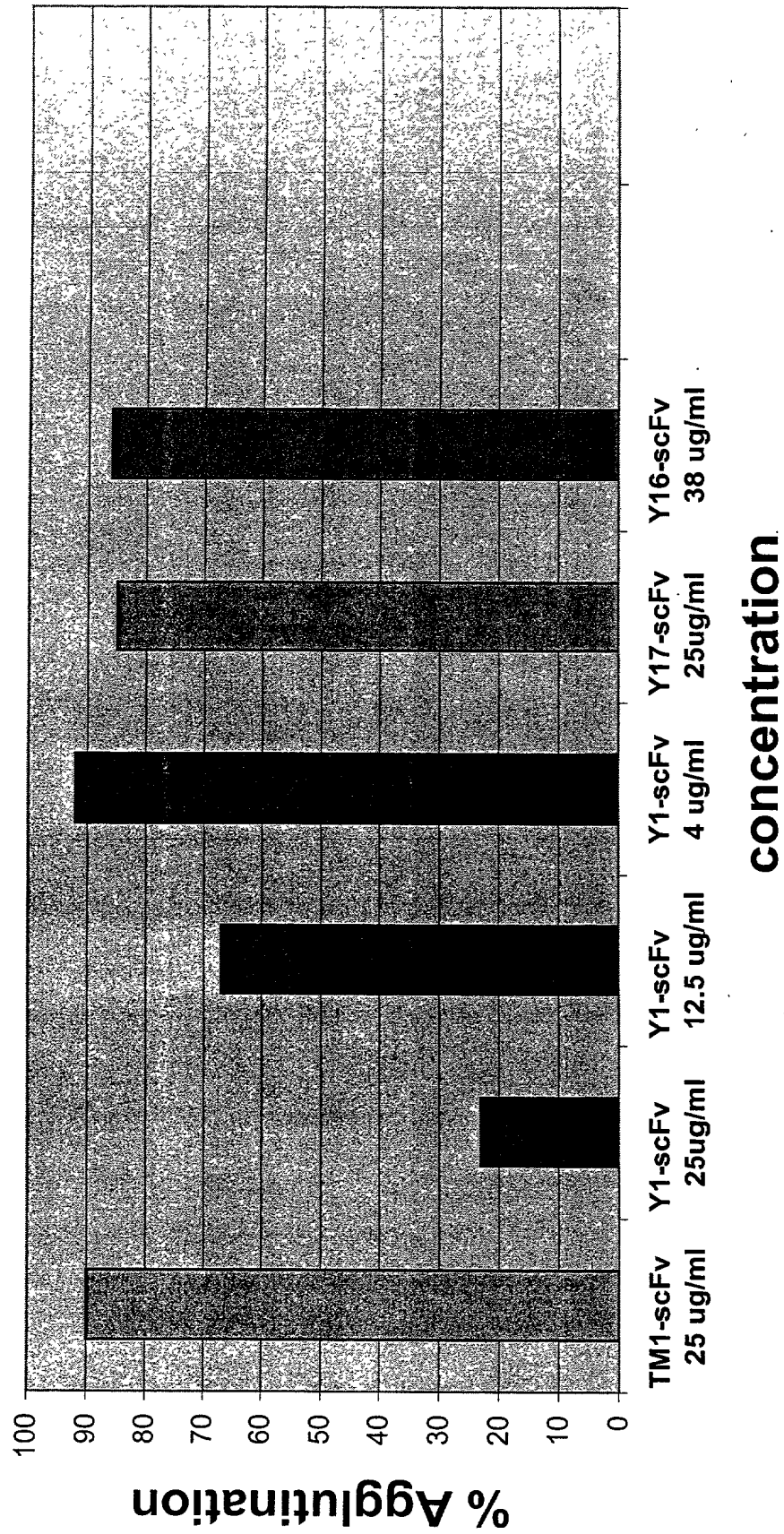


FIG. 16

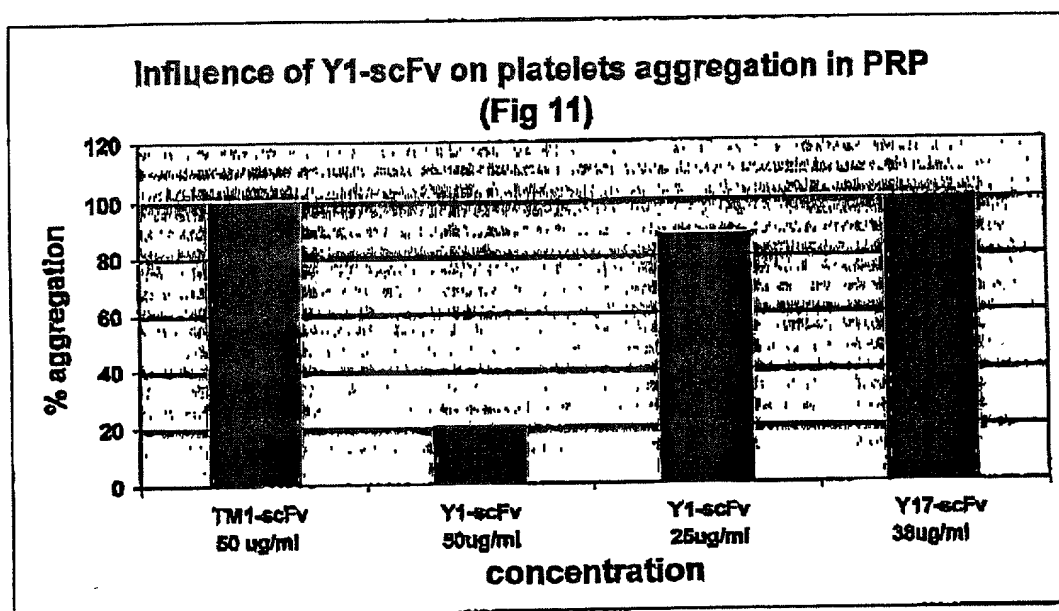


FIG. 17

Induction of platelet agglutination by Y1-IgG in washed platelets

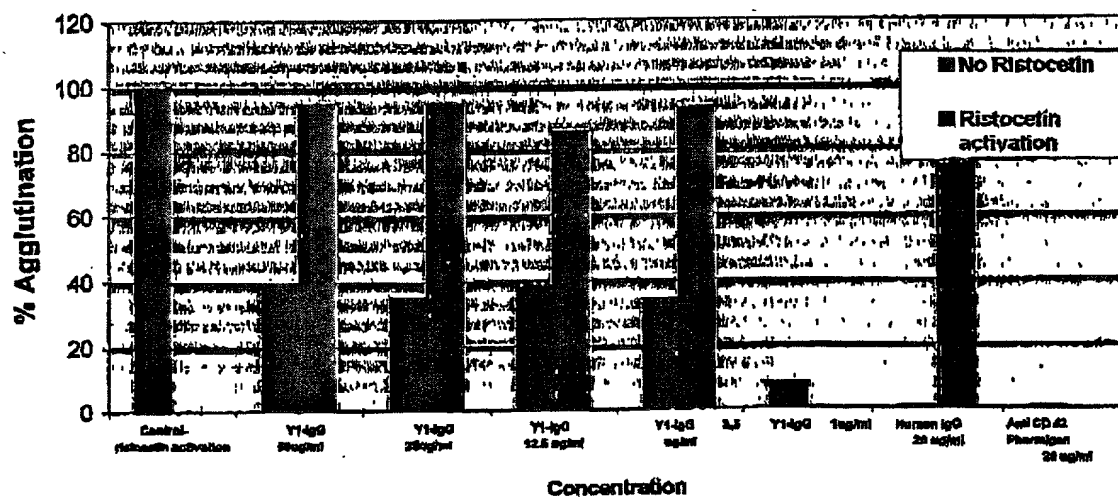


FIG. 18

Induction of platelet aggregation by Y1-IgG in PRP

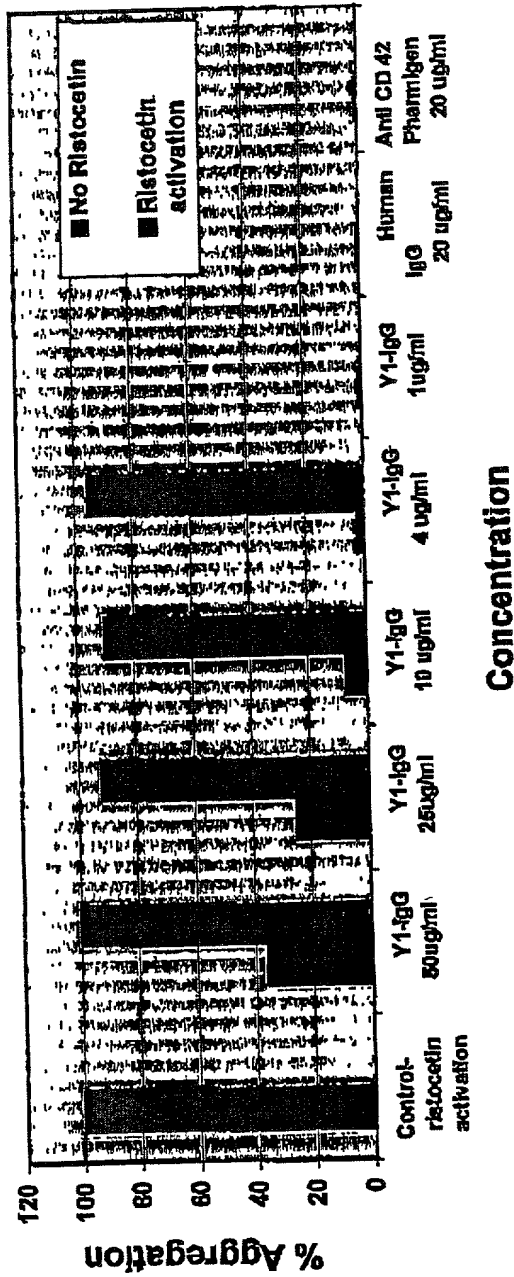
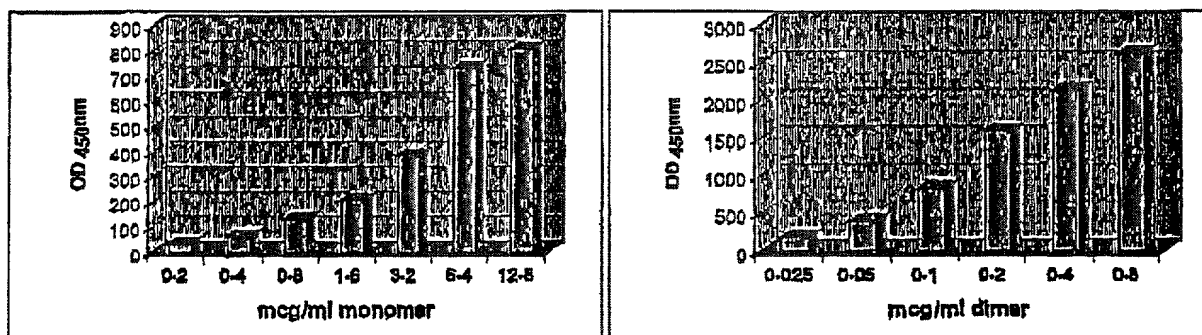


FIG. 19



Specificity of Binding of Y1 and α -CD42 (N1-19) to their Ligands

FIG. 20

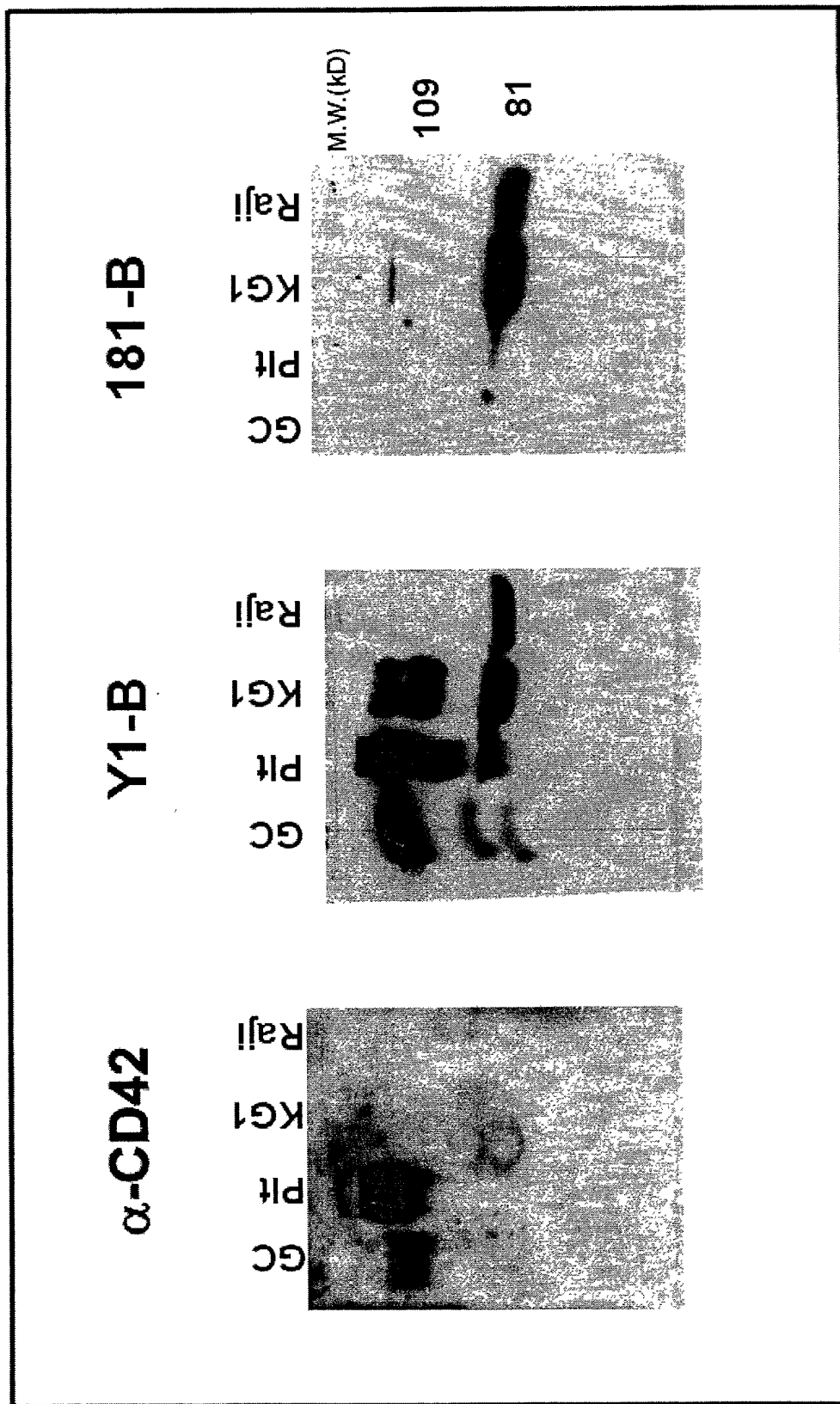
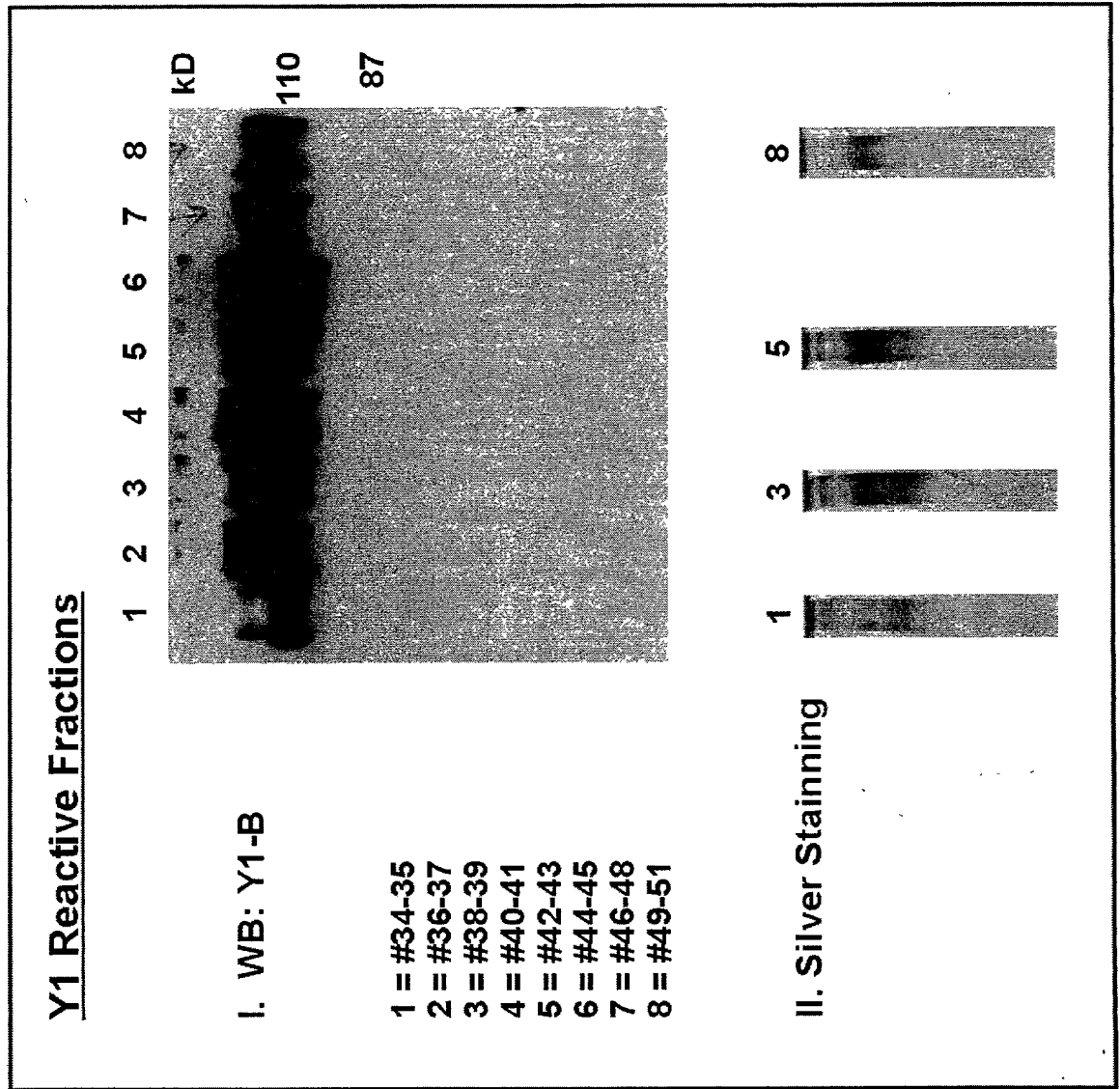


FIG. 21

**Y1-Ligand from KG1 membranes following
Immuno-Precipitation with Y1:
Purification on RP-HPLC**



Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

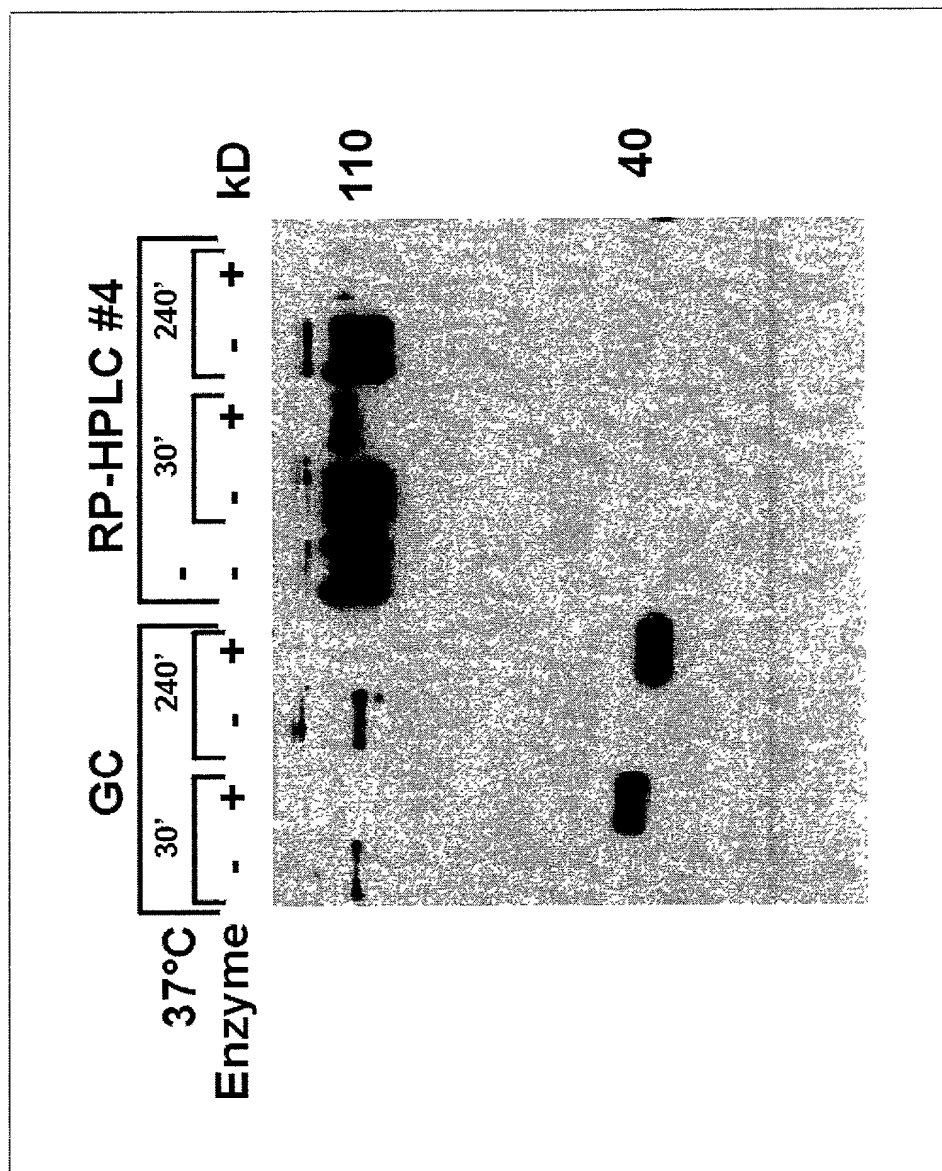
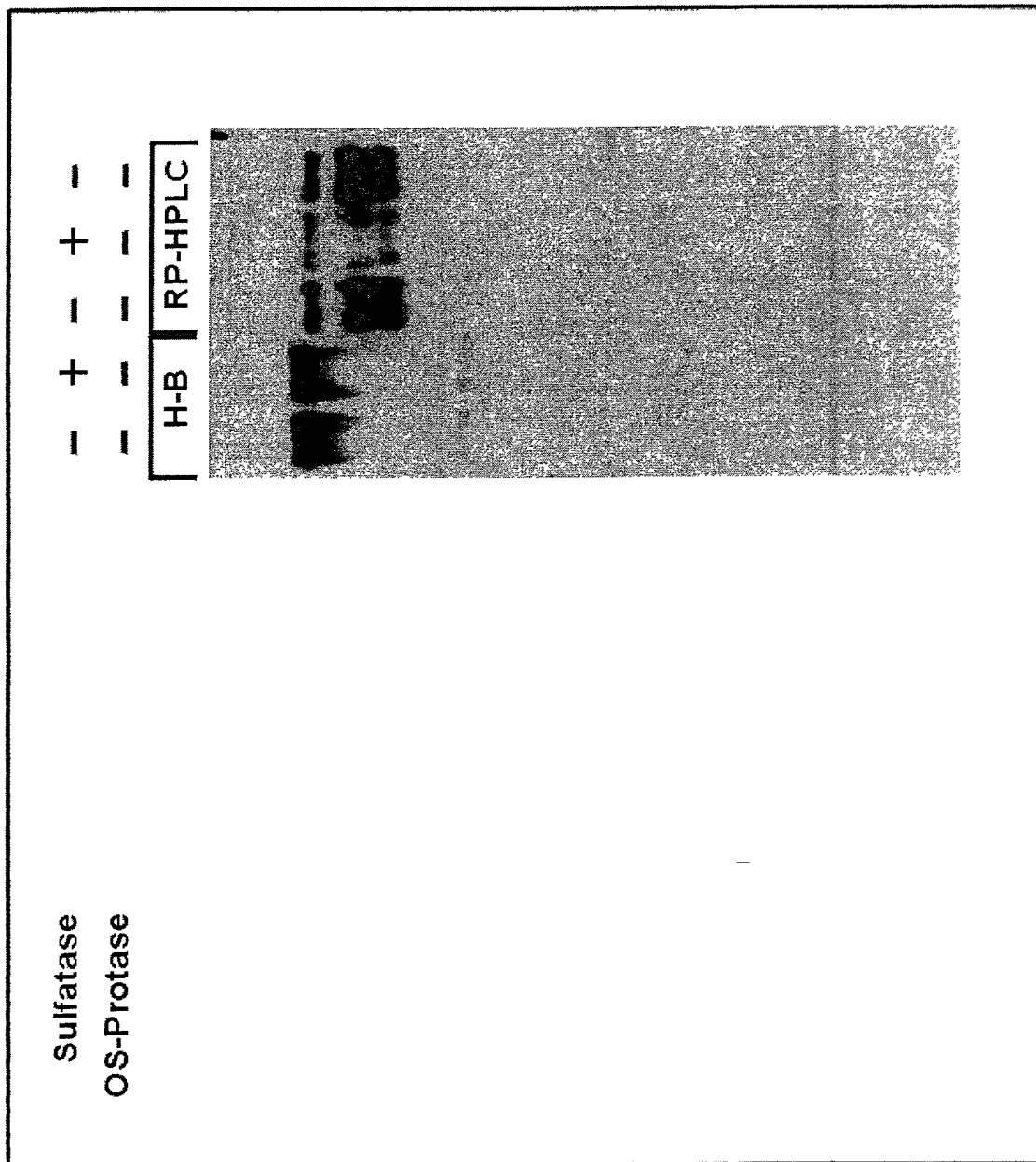


FIG. 22

Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)

FIG. 23



Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1

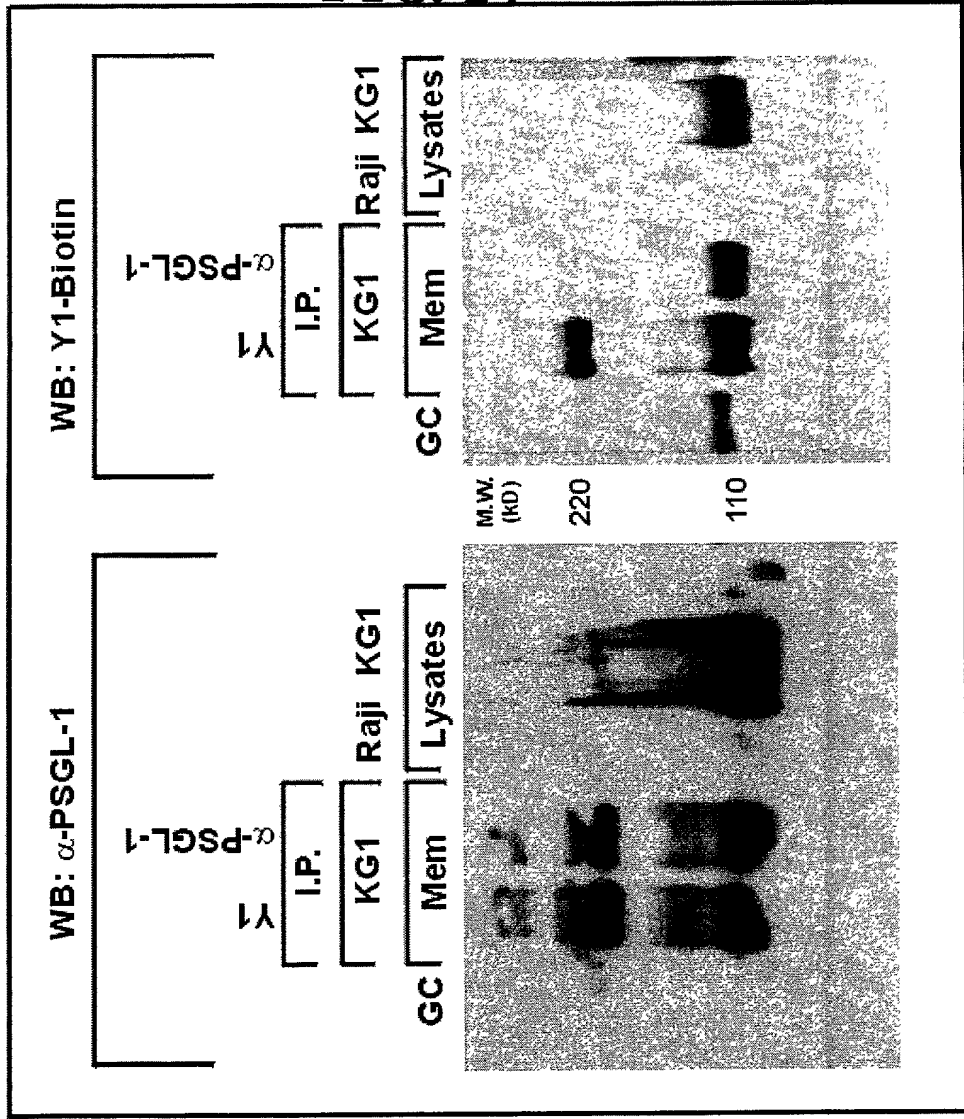
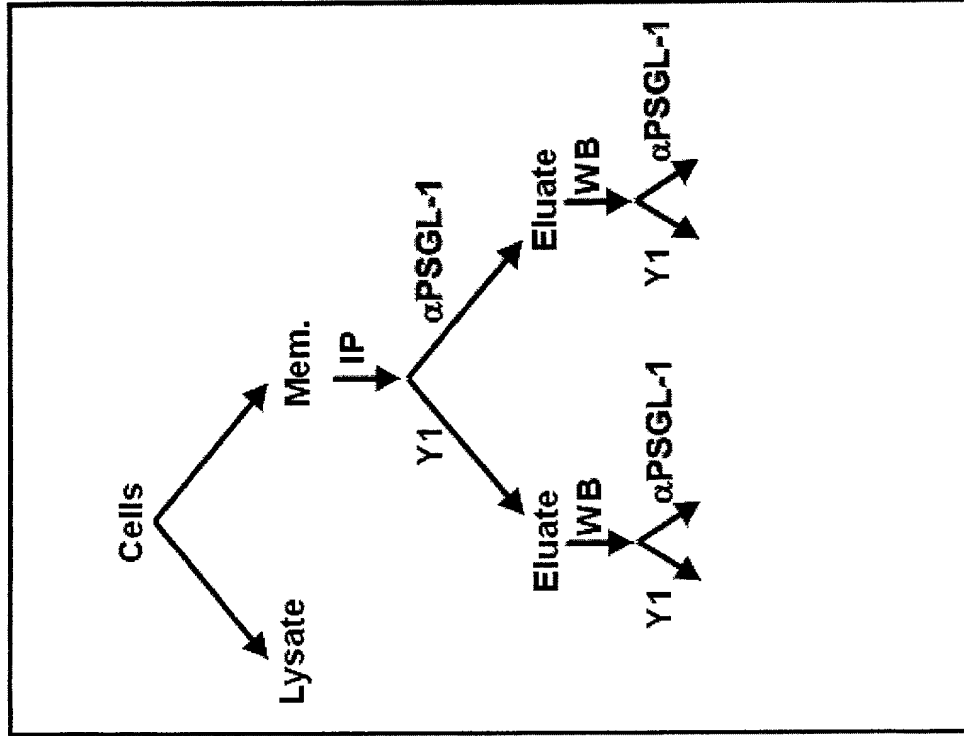
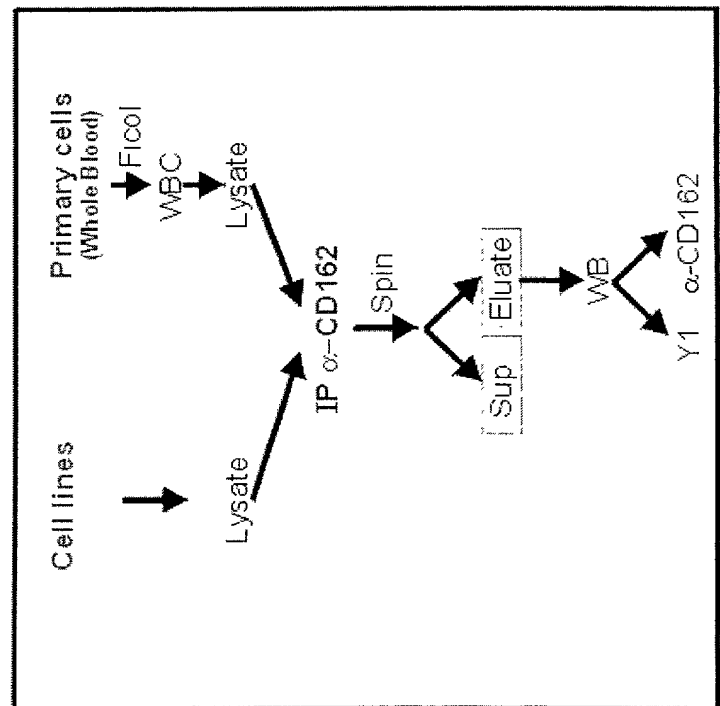


FIG. 24

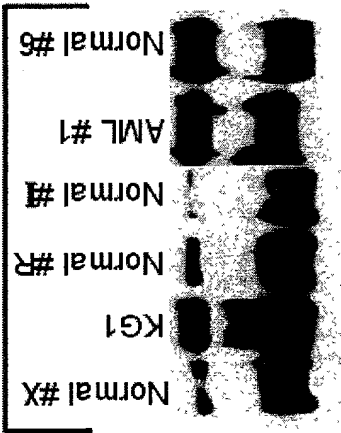
FIG. 25

α -CD162 and Y1:
Comparison between cells
from AML patient and normal
blood



I

WB: α PSGL-1

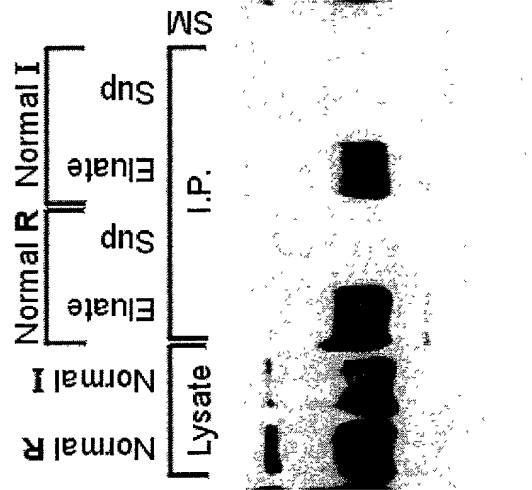


WB: Y1-B



II

WB: α PSGL-1



WB: Y1-B

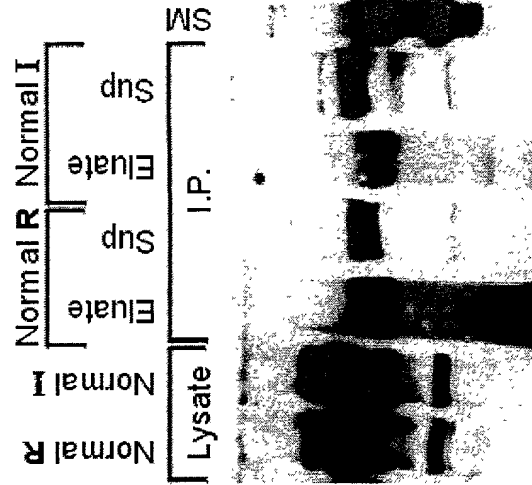
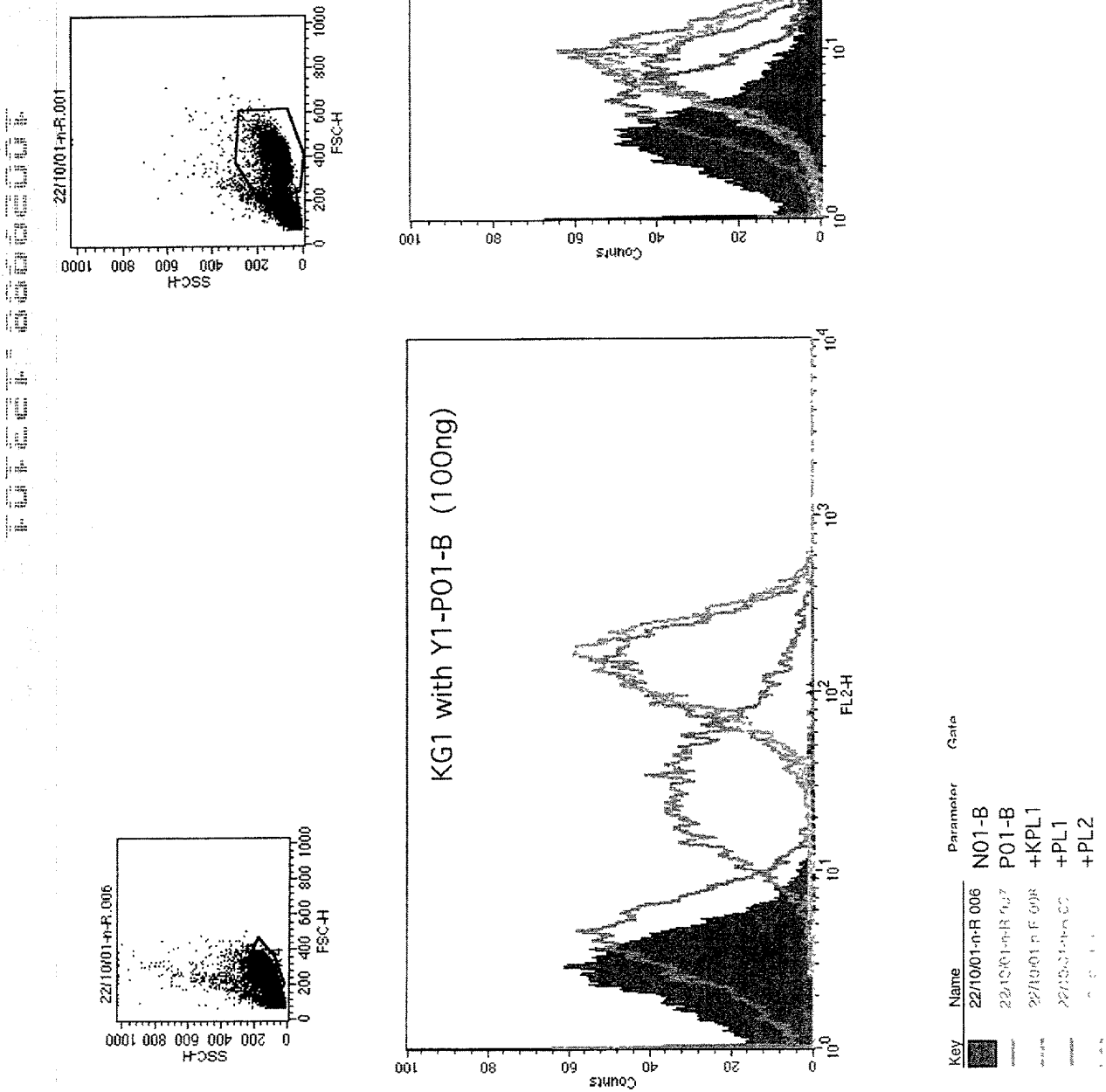
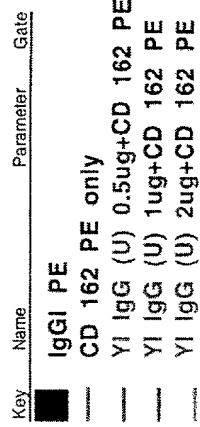


FIG. 26



[illegible]

- FIG. 27**



Specificity of Y1 Binding: Analysis by FACS

- Binding of
Y1-IgG;
competition
with α PSGL-1
(CD162 /KPL1)

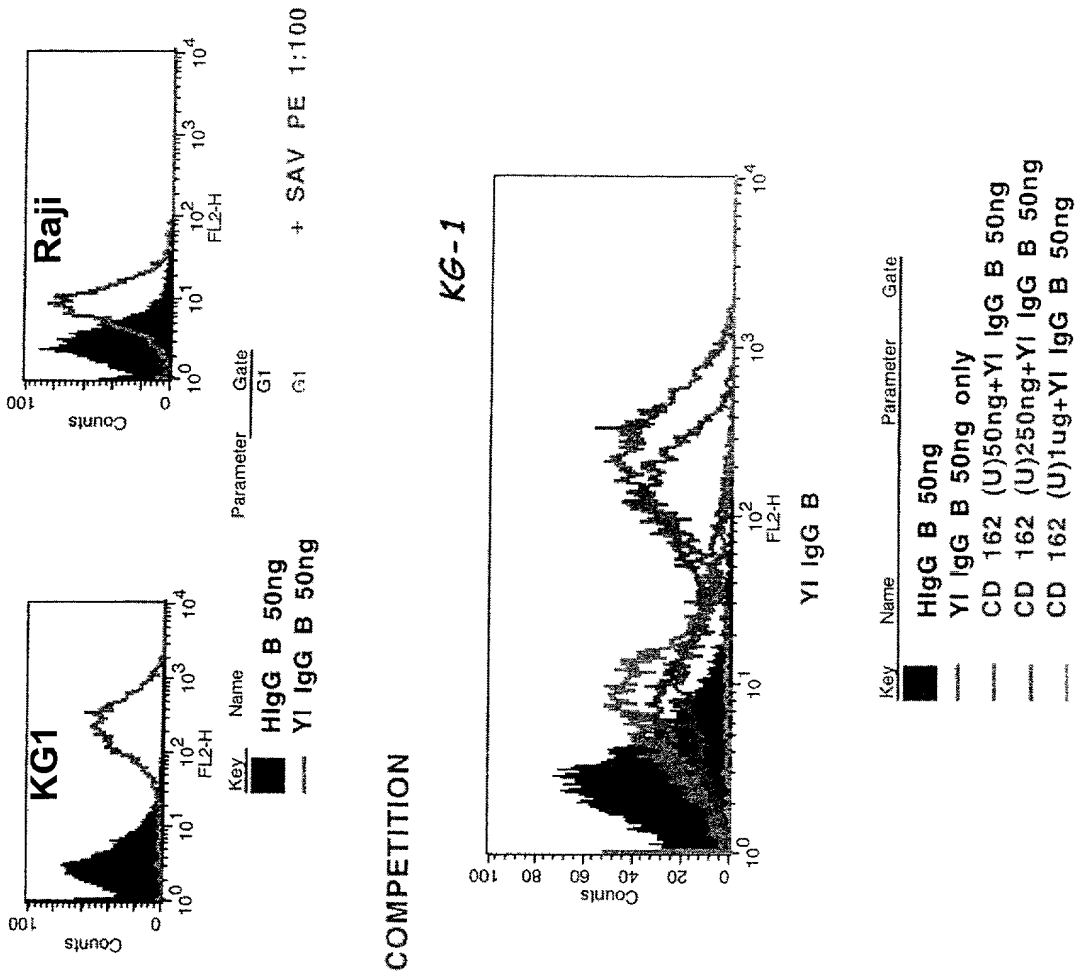


FIG. 28

FIG. 29

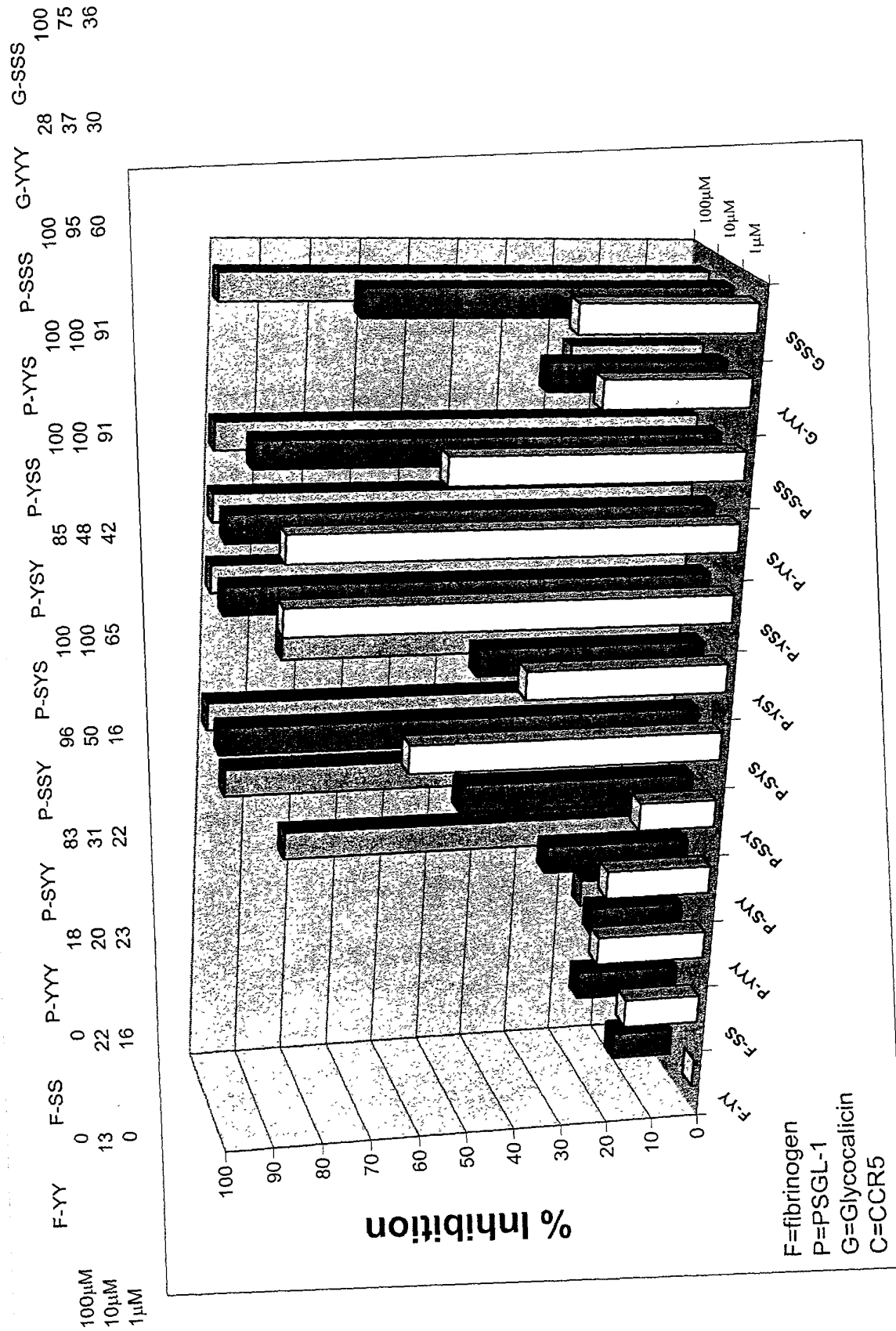


FIG. 30

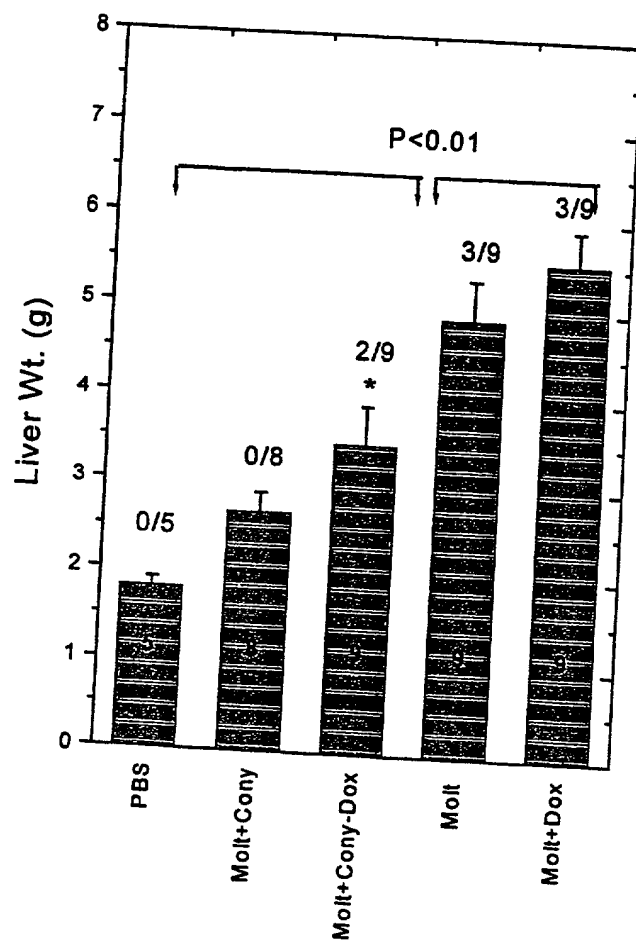


FIG. 31

*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.

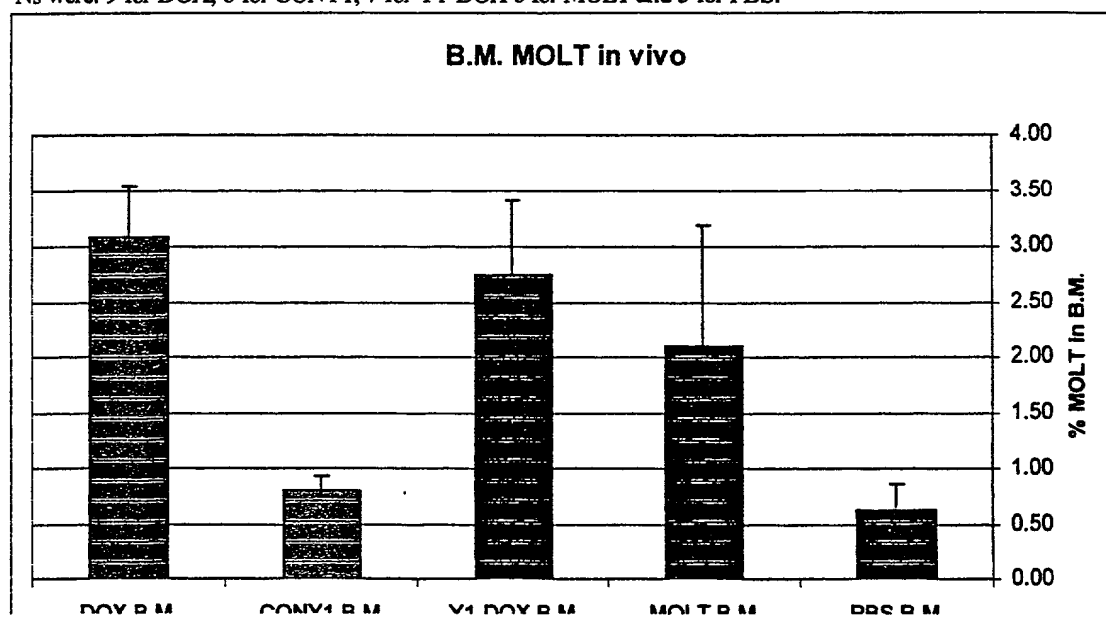
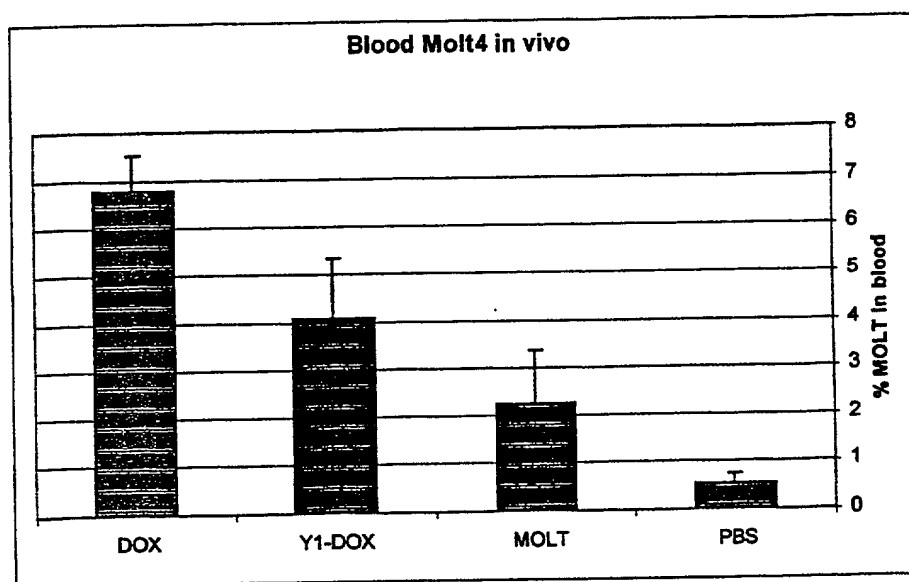


FIG. 32



****Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.**

FIG. 33

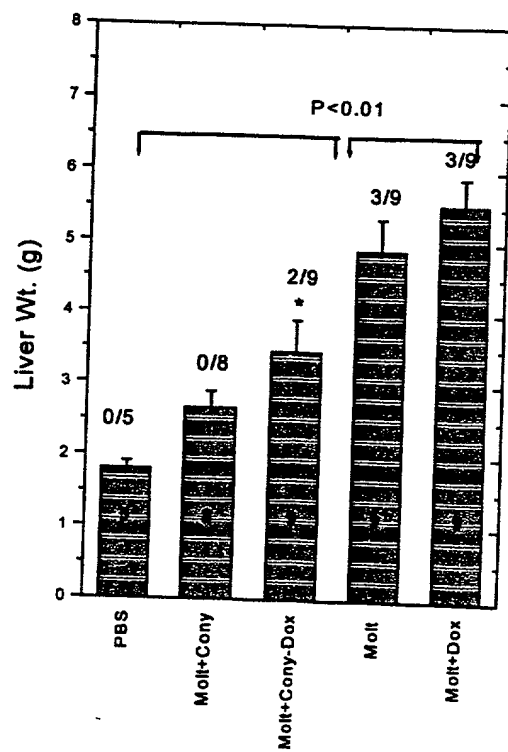


FIG. 34

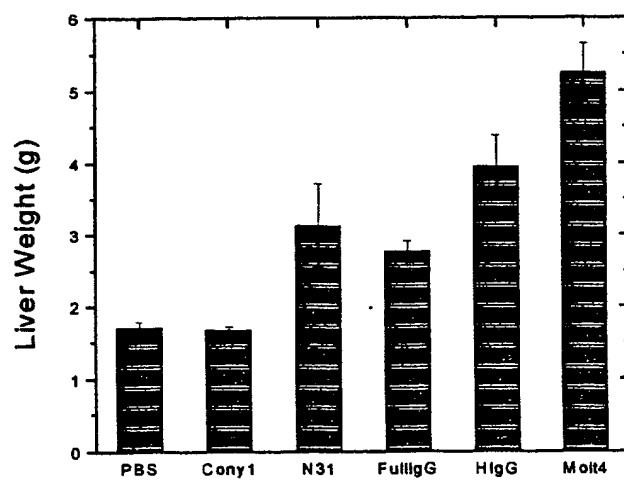


FIG. 35

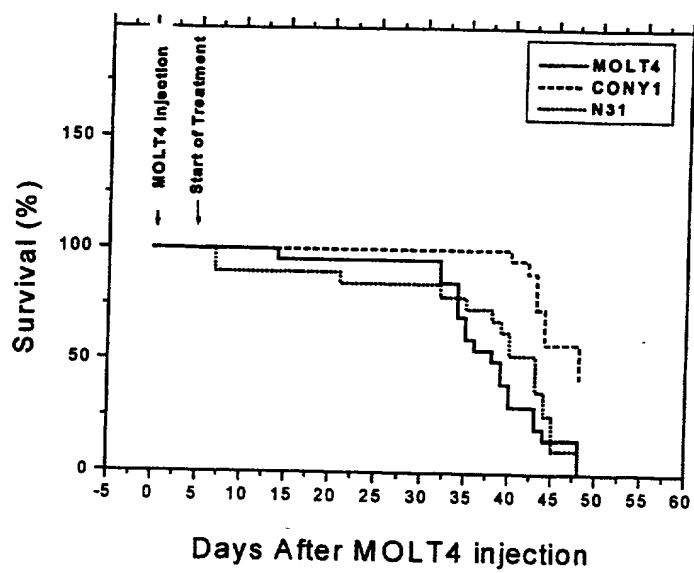


FIG. 36

***Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.

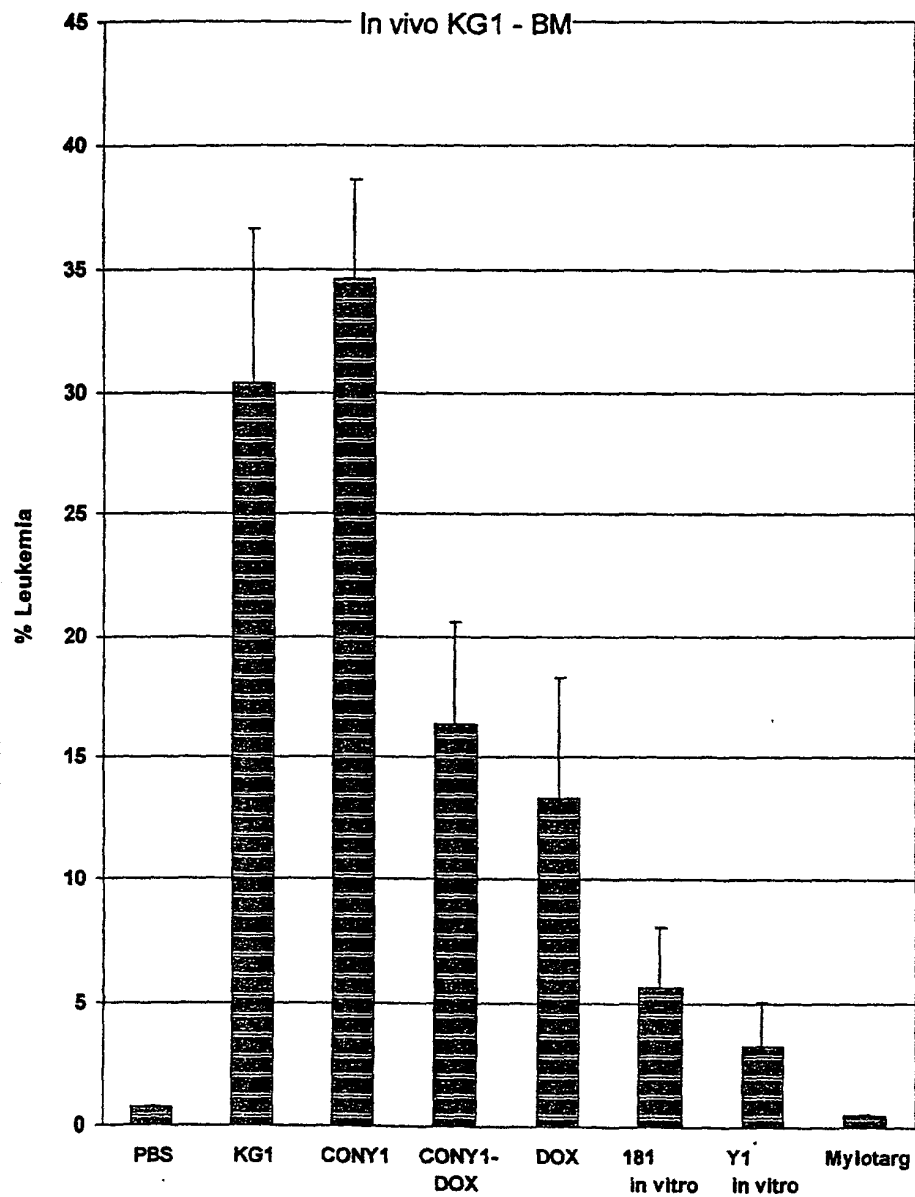
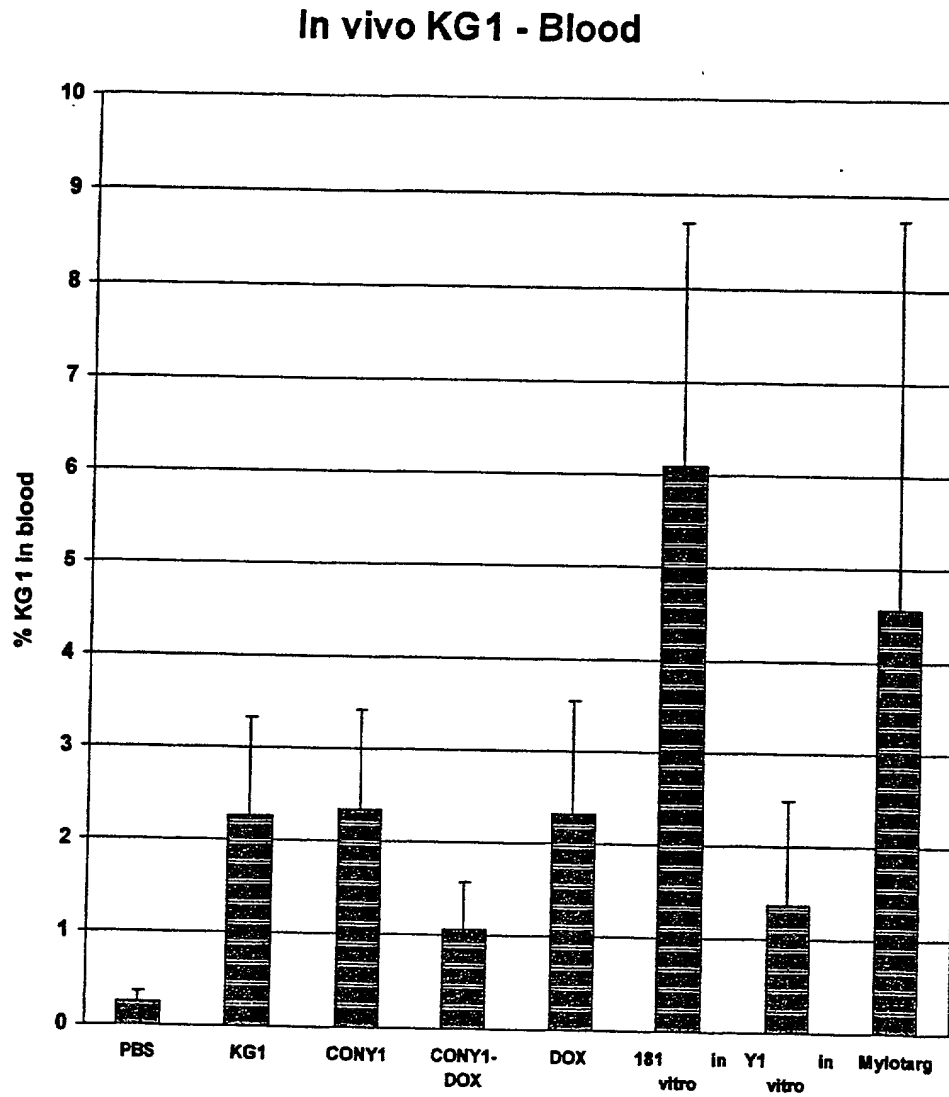


FIG. 37



****Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

FIG. 38

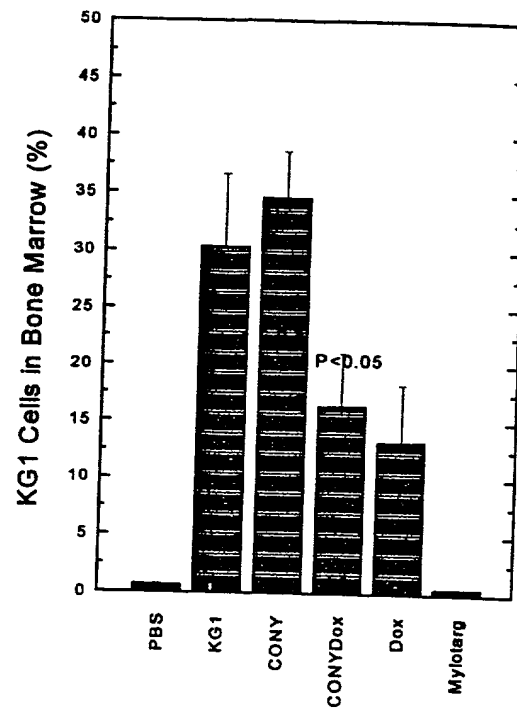
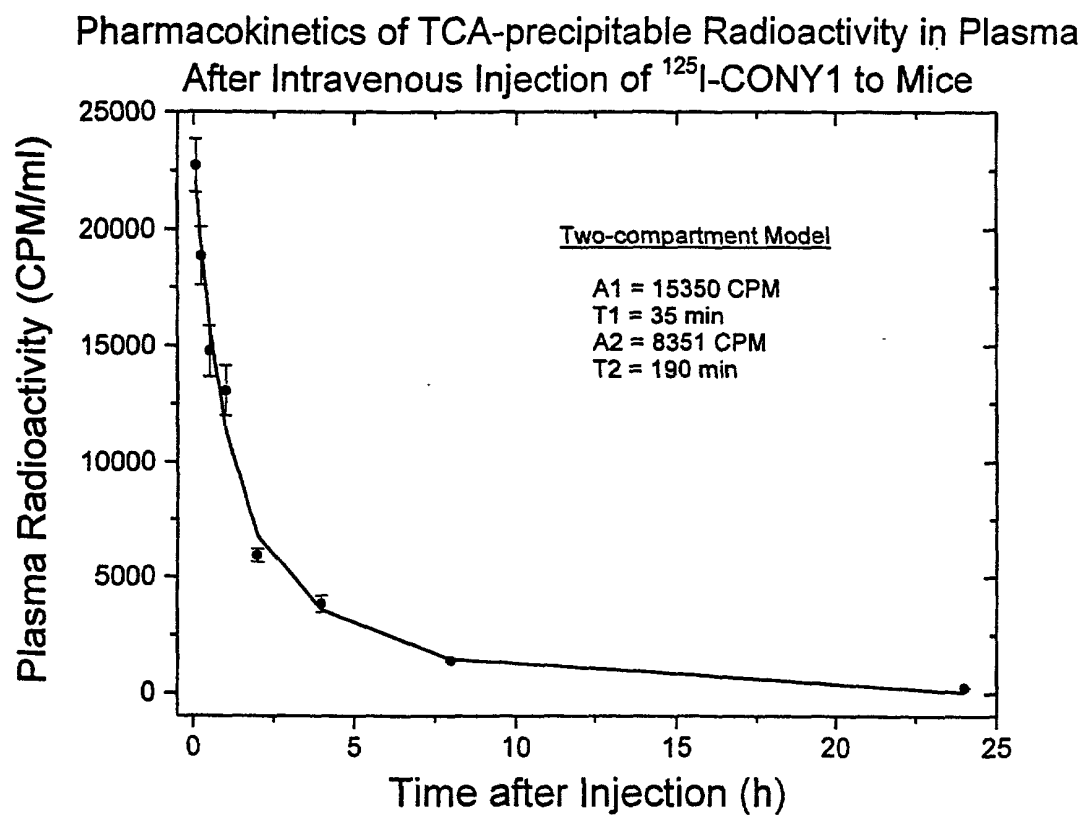


FIG. 39



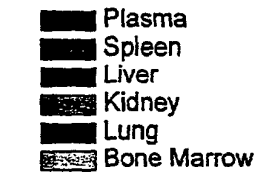
[illegible]

FIG. 41

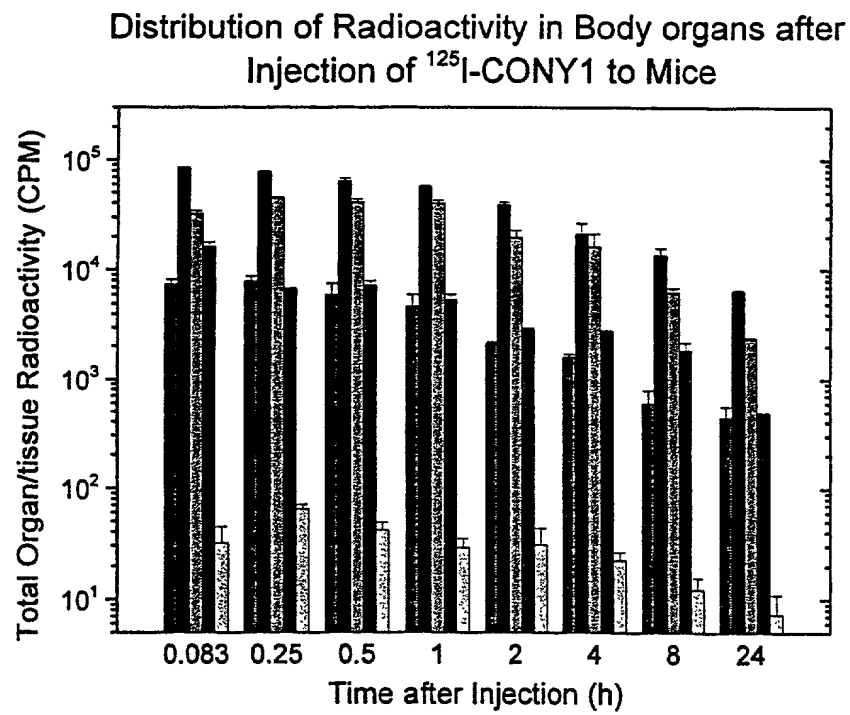


FIG. 42

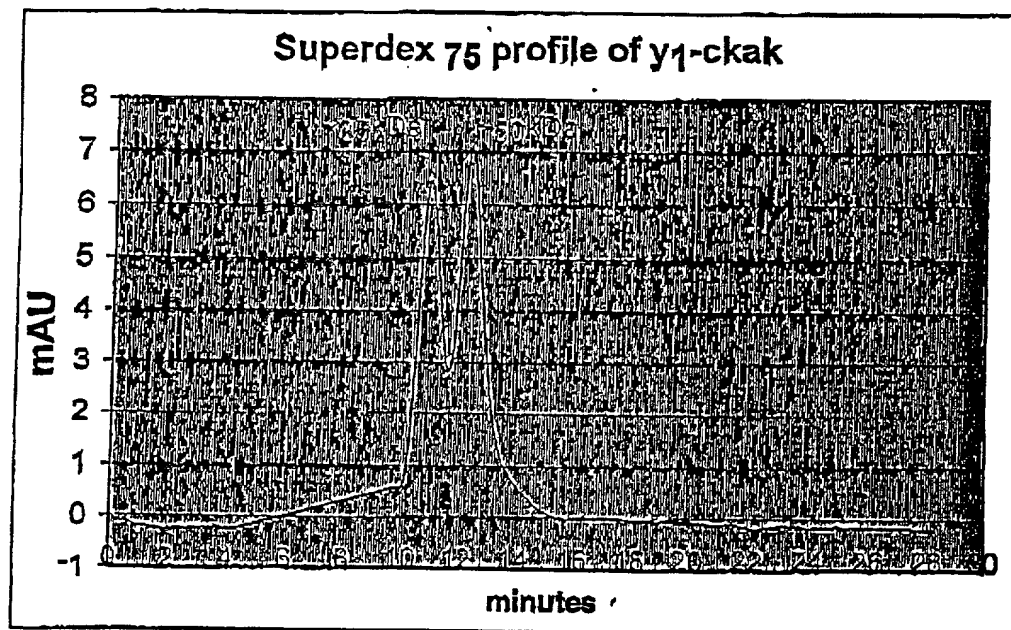


FIG. 43

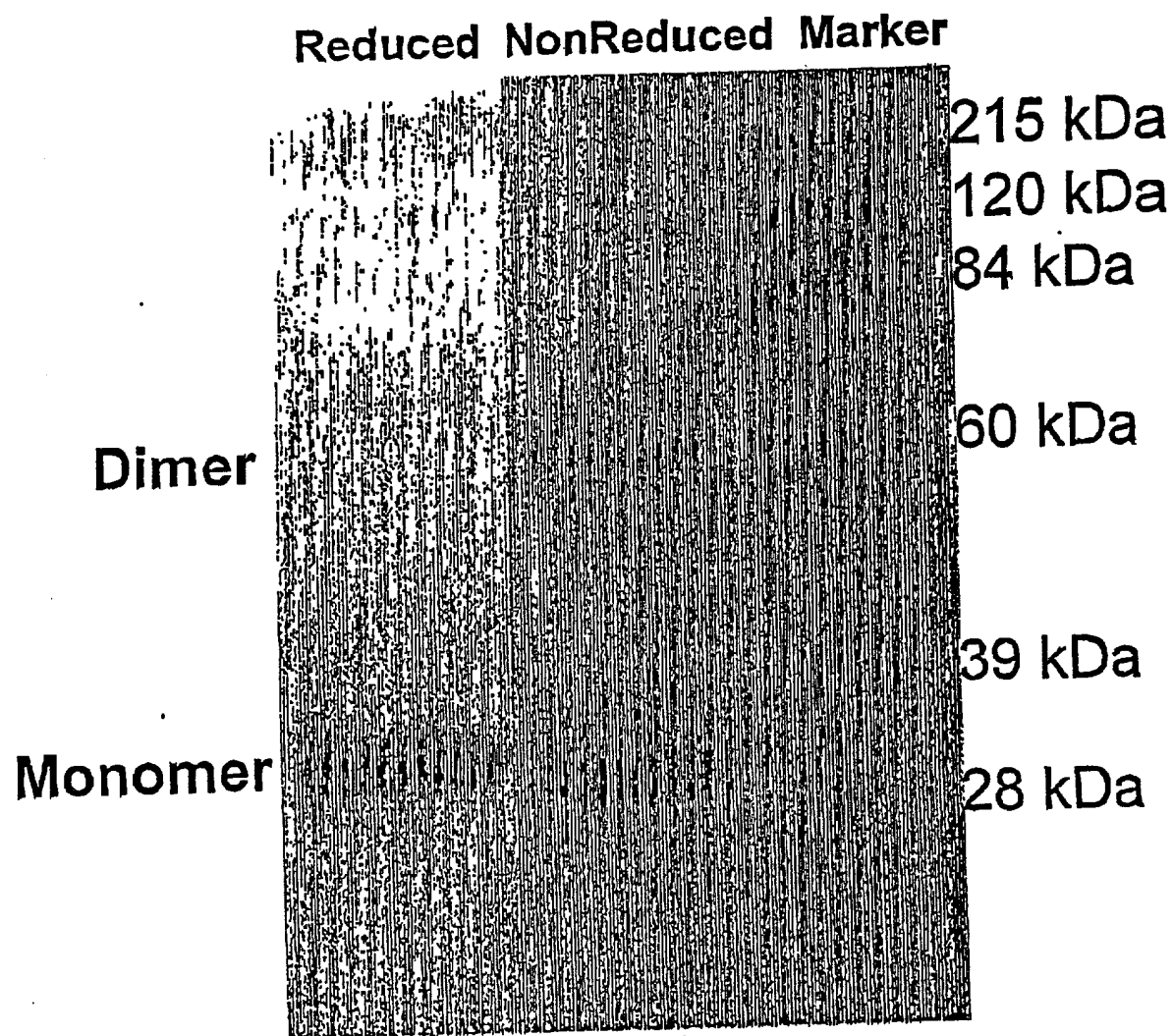
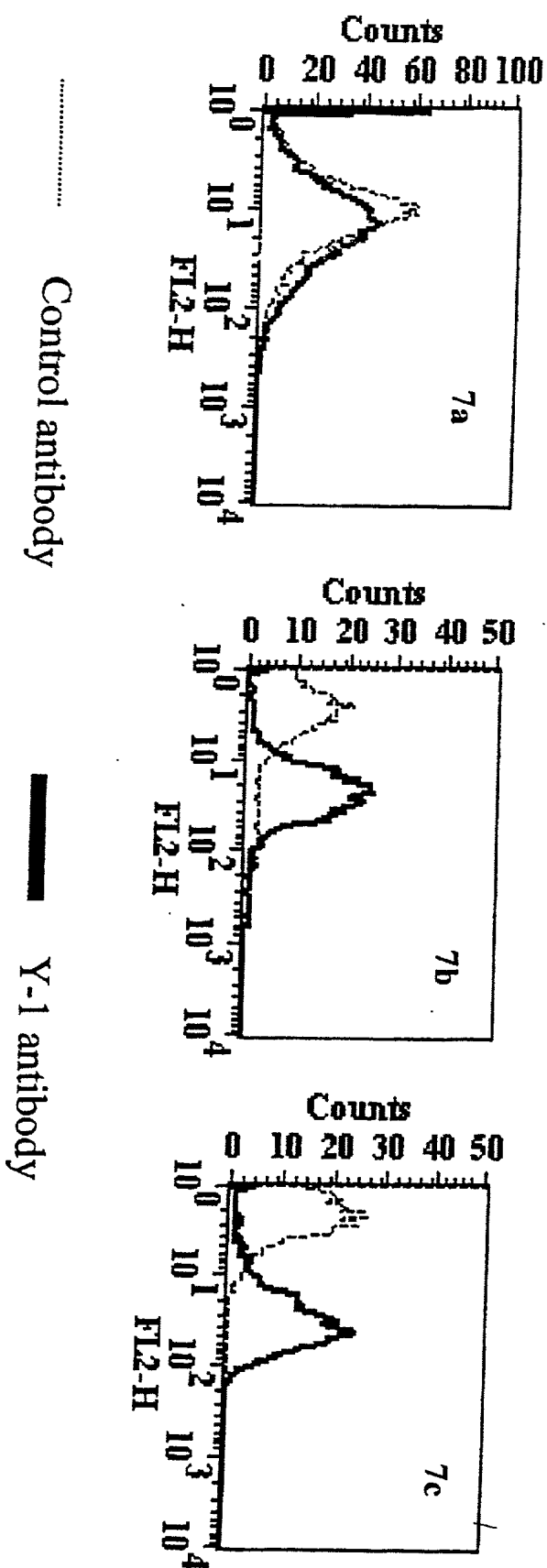


FIG. 44



40030000 4004004

FIG. 45

Epitopes of anti-GPIb α antibodies

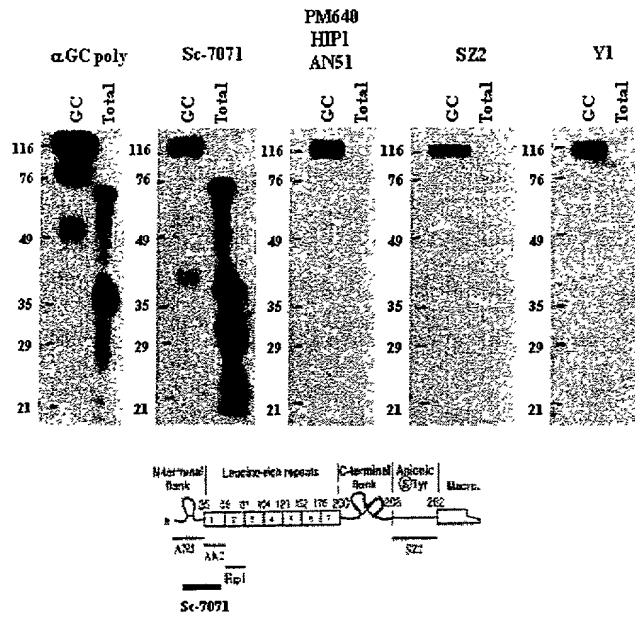


FIG. 46

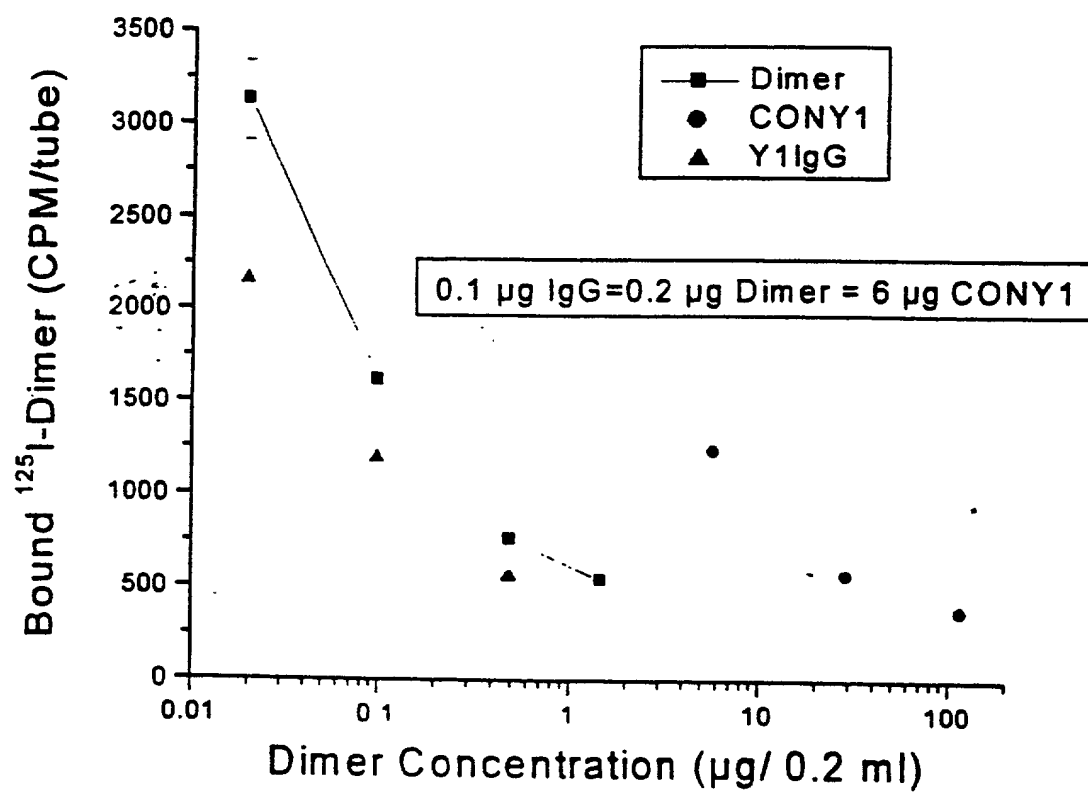


FIG. 47

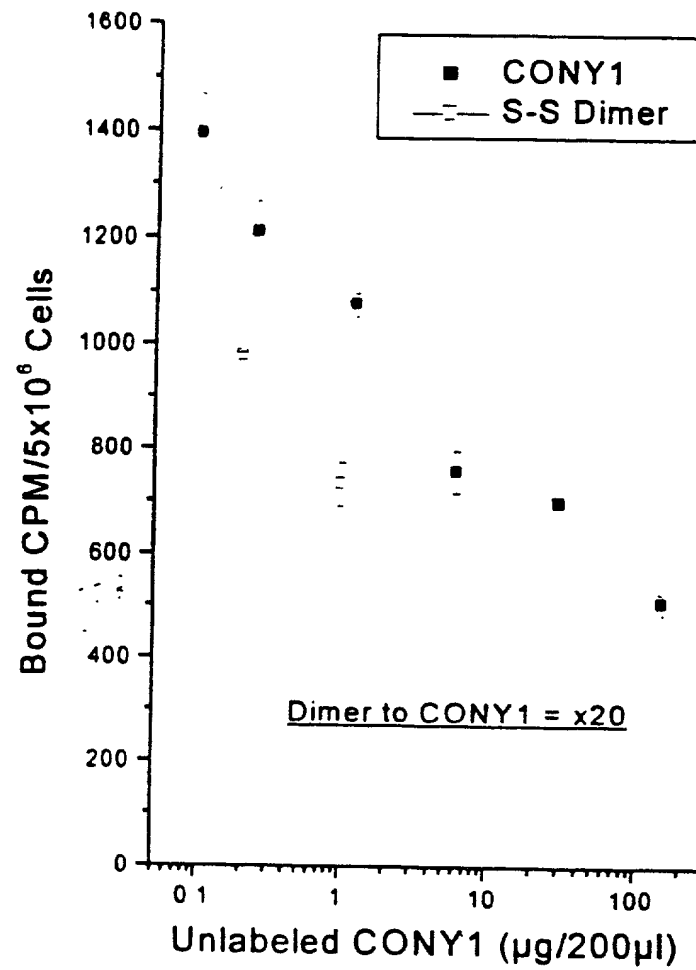


FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

SEQ ID NO: 205 (nucleic acid sequence): SEQ ID NO: 206 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTOACCCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1	<u>M A W A L L L L T L L T Q D T G S W A D</u>
61	ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21	I Q L V E S G G G V V R P G G S L R L S
121	TGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41	C A A S G F T F D D Y G M S W V R Q A P
181	GGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61	G K G L E W V S G I N W N G G S T G Y A
241	GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAACTCCCTGTATCTG
81	D S V K G R F T I S R D N A K N S L Y L
301	CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101	Q M N S L R A E D T A V Y Y C A R M R A
361	CCTGTGATTGTTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGCTTCCACCAAGGGCCCA
121	P V I W G Q G T L V T V S S A S T K G P
421	TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141	S V F P L A P S S K S T S G G T A A L G
481	TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTG
161	C L V K D Y F P E P V T V S W N S G A L
541	ACCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGC
181	T S G V H T F P A V L Q S S G L Y S L S
601	AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201	S V V T V P S S S L G T Q T Y I C N V N
661	CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAC
221	H K P S N T K V D K R V E P K S C D K T
721	CACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACTGTCACTTCTCOTCTTC
241	H T C P P C P A P E L L G G P S V F L F
781	CCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
261	P P K P K D T L M I S R T P E V T C V V
841	GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281	V D V S H E D P E V K F N W Y V D G V E
901	GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301	V H N A K T K P R E E Q Y N S T Y R V V
961	AGCGTCTCACCCTGCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321	S V L T V L H Q D W L N G K E Y K C K V
1021	TCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC
341	S N K A L P A P I E K T I S K A K G Q P
1081	OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
361	R E P Q V Y T L P P S R E E M T K N Q V
1141	AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381	S L T C L V K G F Y P S D I A V E W E S
1201	AATGGGCAGCCGGAGAACAACACTACAAGACCACGTCTCCCGTGCTGGACTCCGACGGCTCC
401	N G Q P E N N Y K T T S P V L D S D G S
1261	TTCTTCCTCTATAGCAAGCTCACCGTGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421	F F L Y S K L T V D K S R W Q Q G N V F
1321	TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441	S C S V M H E A L H N H Y T Q K S L S L
1381	TCTCTGGGTAAATGA
461	S L G K *

FIG. 48B: The ORF and Amino Acid Sequence of Y1-LC

SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

```
1      ATGGCCTGGGCTCTGCTGCTCCTCACCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1      M  A  W  A  L  L  L  L  T  L  L  T  Q  D  T  G  S  W  A  D
61     GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21     A  E  L  T  Q  D  P  A  V  S  V  A  L  G  Q  T  V  R  I  T
1212   TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41     C  Q  G  D  S  L  R  S  Y  Y  A  S  W  Y  Q  Q  K  P  G  Q
181    GCCCCTGTACTTGTCTATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
161    A  P  V  L  V  I  Y  G  K  N  N  R  P  S  G  I  P  D  R  F
241    TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81     S  G  S  S  S  G  N  T  A  S  L  T  I  T  G  A  Q  A  E  D
301    GAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGA
101    E  A  D  Y  Y  C  N  S  R  D  S  S  G  N  H  V  V  F  G  G
361    GGGACCAAGCTGACCGTCCTAGGTCAAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121    G  T  K  L  T  V  L  G  Q  P  K  A  A  P  S  V  T  L  F  P
421    CCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCCACTGGTGTGTCTCATAAGTGACTTC
141    P  S  S  E  E  L  Q  A  N  K  A  T  L  V  C  L  I  S  D  F
481    TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161    Y  P  G  A  V  T  V  A  W  K  A  D  S  S  P  V  K  A  G  V
541    GAGACCACCACACCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181    E  T  T  T  P  S  K  Q  S  N  N  K  Y  A  A  S  S  Y  L  S
601    CTGACGCCTGAGCAGTGGGAAGTCCCACAAAAGCTACAGCTGCCAGGTACGCATGAAGGG
201    L  T  P  E  Q  W  K  S  H  K  S  Y  S  C  Q  V  T  H  E  G
661    AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATGA
221    S  T  V  E  K  T  V  A  P  T  E  C  S  *
```

FIG. 49

	1	11	21	31	41	51	
1	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA	ISGSGGSTYY	60
61	ADSVKGRFTI	SRDNSKNTLY	LQMNSLRAED	TAVYYCARVA	KTLMRQYSLW	GQGTLVTVSR	120
121	GGGSGGGGGS	GGGGSSELTQ	DPAVSVALGQ	TVRITCQGDS	LRSYVASWYQ	QKPGQAPVLV	180
181	IYGKNNRPSG	IFDRFSGSSS	GNTASLTITG	AQAEDEADYY	CNSRDSSGNH	VVFGGGTKLT	240
241	VLGAAAEQKL	ISEEDLNGAA					

EVQLVESGGG

FIG. 50

		10	20	30	40	50	60
1		AtTaTTAcTc	gCGGCCcAGC	CgGCCcAGC	CGAGGTGCAG	CTGGTGGAGT	CTGGGGGAGG
3		L L L A A Q P A M A	E V Q L V E S G G G				
		70	80	90	100	110	120
1		CTTGGTACAG	CCTGGGGGGT	CCCTGAGACT	CTCCTGTGCA	GCCTCTGGAT	TCACCTTAG
3		L V Q P G G S L R L S C A A S G F T F S					
		130	140	150	160	170	180
1		CAGCTATGCC	ATGAGCTGGG	TCCGCCAGGC	TCCAGGGAAG	GGGCTGGAGT	GGGTCTCAGC
3		S Y A M S W V R Q A P G K G L E W V S A					
		190	200	210	220	230	240
1		TATTAGTGGT	AGTGGTGGTA	GCACATACTA	CGCAGACTCC	GTGAAGGGCC	GGTTCACCAT
3		I S G S G G S T Y Y A D S V K G R F T I					
		250	260	270	280	290	300
1		CTCCAGAGAC	AATTCCAAGA	ACACGCTGTA	TCTGCAAATG	AACAGCCTGA	GAGCCGAGGA
3		S R D N S K N T L Y L Q M N S L R A E D					
		310	320	330	340	350	360
1		CACGGCCGTG	TATTACTGTG	CAAGAGCGG	GCAGAGTATT	AAGCGTATAT	GGGCGCAAGG
3		T A V Y Y C A R T G Q S I K R S W G Q G					
		370	380	390	400	410	420
1		TACCCGTGGT	ACCGTGTGCA	GAGGTGGAGG	CGGTTCAgGC	GGAgGTGgCT	CTGGCGGTGG
3		T L V T V S R G G G G S G G G G S G G G					
		430	440	450	460	470	480
1		CGGATCGTCT	GAgCTGACTC	AGGACCCTGC	TGTGTCTGTG	GcCTTGGGAC	AgACAGTCAG
3		G S S E L T Q D P A V S V A L G Q T V R					
		490	500	510	520	530	540
1		GATcACATGC	CAAGGAgACA	GCCTCAGAAG	CTATTATGCA	AGCTGGTACC	AGCAGAAGCC
3		I T C Q G D S L R S Y Y A S W Y Q Q K P					
		550	560	570	580	590	600
1		AGGACAGGCC	CCTGTACTTG	TCATCTATGG	TAAAAACAAC	CGGCCCTCAG	GGATCCCAGA
3		G Q A P V L V I Y G K N N R P S G I P D					
		610	620	630	640	650	660
1		CCGATTCTCT	GGCTCCAGCT	CAGGAAACAC	AGCTTCCTTG	ACCATCACTG	GGGCTCAGGC
3		R F S G S S S G N T A S L T I T G A Q A					
		670	680	690	700	710	720
1		GGAAGATGAG	GCTGACTATT	ACTGTAACTC	CCGGGACAGC	AGTGGTAACC	ATGTGGTATT
3		E D E A D Y Y C N S R D S S G N H V V F					
		730	740	750	760	770	780
1		CGGCGGAGGG	ACCAAGCTGA	CCGTCTAGG	TGCGGCCGCA	GAACAAAAAC	TCATCTCAGA
3		G G G T K L T V L G A A A E Q K L I S E					
		790	800	810	820	830	840
1		AGAgGAtCTG	AatGGGGCCG	CAGGAACTG	TtGAATTTT	TAAGTTTAcC	T
3		E D L N G A A * N C * I F * V N					

Y16 SEQ ID NO: 210

FIG. 51

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTT DDYGMSWVRQ
41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL
81 YLQMNSLRAE DTAVYYCARM RAPVIWGQGT LTVSRGGGG
121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY
161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA
201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG
241 GGLNDIFEAQ KIEWHE

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

[illegible]

Y1-cys-kak scFv (SEQ ID NO. 212)

I MEVQLVESGG GVVPRPGGSLR LSCAASGFTF DDYGMSWVRQ
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK